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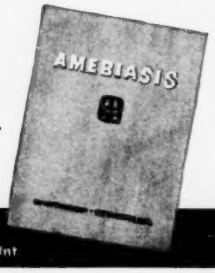

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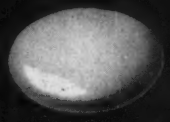

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STUDIES OF NIACIN REQUIREMENT IN MAN. I. EXPERIMENTAL PELLAGRA IN SUBJECTS ON CORN DIETS LOW IN NIACIN AND TRYPTOPHAN [1]

By GRACE A. GOLDSMITH, HERBERT P. SARETT, U. D. REGISTER, AND JANIS GIBBENS

(From the Nutrition Research Laboratory, Departments of Medicine and Biochemistry, Tulane University School of Medicine, and the Charity Hospital of Louisiana at New Orleans, La.)

(Submitted for publication January 11, 1952; accepted March 24, 1952)

Early attempts to estimate human niacin requirement were based on calculations of the niacin content of pellagragenic and nonpellagragenic diets (1, 2). It was appreciated that the protein content of the diet was in some way related to niacin requirement. Recent studies showing that the amino acid tryptophan is converted in part to niacin compounds in man (3–5) may explain the above relationship, and also the pellagra preventive effect of foods which are low in niacin but rich in "good" protein.

The present experiments were designed to study niacin requirement in man with diets of known tryptophan content. Estimation of minimum tryptophan requirement for nitrogen balance (6) permits formulation of diets containing little excess tryptophan which may be converted to niacin. The discovery of the major metabolites of niacin, namely N^1-methylnicotinamide (N^1-Me) and the 6-pyridone of N^1-methylnicotinamide (pyridone), and the development of methods for the measurement of these compounds in urine, now permit more complete studies of niacin metabolism (7–11).

Seven subjects were maintained on diets low in niacin and tryptophan for from 40 to 135 days and the urinary excretion of niacin metabolites was determined. Clinical signs of pellagra developed in the three subjects who remained on one of these diets for more than 50 days.

METHODS

The seven subjects were white females, 25 to 54 years of age, who were found to be essentially free of organic disease and who had some type of psychoneurosis. They were maintained in a metabolism ward and were ambulatory throughout the experimental period. Frequent clinical examinations were carried out for any signs which might suggest nutritional deficiency. Basal diets were carefully weighed and administered under strict supervision.

The diet for two complete days was analyzed at intervals for niacin, tryptophan and total nitrogen. The diet for each meal was blended with water in a Waring blendor to give a total volume of 1,000 to 1,500 ml. Aliquots of this material were autoclaved in 1 *N* sulfuric acid solution for 45 minutes, neutralized and analyzed for niacin microbiologically, using *L. arabinosus* (12). This organism was also employed for tryptophan analysis using aliquots of the homogenate which had been autoclaved in 5 *N* sodium hydroxide in the presence of cysteine (13). For these analyses 15 ml. portions of 8 *N* sodium hydroxide solution were placed in 125 ml. Erlenmeyer flasks and 400 mg. of cysteine and 10 ml. of the blended diet were rapidly added. These were covered at once with beakers and autoclaved for six hours at 121° C. After neutralization with hydrochloric acid, the solutions were diluted to 100 ml., filtered and analyzed. Under these conditions it is assumed that all tryptophan liberated from the proteins was racemized and was present as D,L-tryptophan. Although it is possible that some tryptophan in the proteins may have been destroyed by this procedure, L- or D,L-tryptophan added to the samples was not destroyed and treatment of L- or D,L-tryptophan alone with these reagents resulted in 90 to 100% recovery. Analyses of the diets and of individual foods gave reproducible results. Nitrogen was determined by the macro-Kjeldahl method.

All urine was collected for 24 hour periods in dark bottles containing 5 ml. of glacial acetic acid and kept in the refrigerator until pooled for analysis. In long term experiments, it was found desirable to pool the urine in four day periods. A portion of each pooled urine was neutralized to pH 6.9, and stored in the frozen state in several 16 × 150 mm. screw cap tubes until analyzed. Microbiological methods were used for determination of niacin (12), quinolinic acid (14) and tryptophan (5). Fluorometric methods for determination of N^1-methylnicotinamide (N^1-Me) (10) and the 6-pyridone of N^1-Me (pyridone) (11) were modified for use in this laboratory. The procedure for measurement of N^1-Me is

[1] This work was supported by grants from the Nutrition Foundation, Inc., The Roche Anniversary Foundation, the Williams-Waterman Fund of the Research Corporation, and the Division of Research Grants and Fellowships of the National Institutes of Health, United States Public Health Service.

TABLE I

*Corn diet low in niacin and tryptophan**

Breakfast	gm.
Orange or grapefruit juice	200
Corn grits (dry weight)	50
White bread	30
Oleomargarine	20
Sugar	20
Dinner	
Mor beef	20
Rice (dry weight)	33
Beets or carrots†	100
Corn bread	75
Oleomargarine	20
Fruit cocktail or apple sauce†	150
Apple juice	200
Supper	
Green beans or mustard greens†	100 50
Corn bread	25
Oleomargarine	10
Grape juice	100
Sugar	30
Gelatin	20
Pears or pineapple†	100
Between Meals	
Lemon juice	30
Sugar	10
Cookies	40
Prune juice	100

* The unenriched white flour (Gold Medal) was generously furnished by General Mills, Inc., through the courtesy of Dr. H. S. Faulkner. The unenriched corn meal and grits were generously provided by the Quaker Oats Company, through the courtesy of Dr. F. N. Peters. Mor Beef was kindly supplied by Wilson and Company, Inc., through the courtesy of Dr. E. J. Czarnetzky.

† Canned, drained weight, given alternately.

White flour and corn products were unenriched.

Analysis of diet: nitrogen 7.0 gm., niacin 4.7 mg., tryptophan 190 mg.

Energy value: approximately 2,000 calories.

sufficiently sensitive to detect excretion as low as 0.5 mg. daily and at this level, values are reproducible to within 20%. The method for estimation of the pyridone is much less sensitive and may fail to detect excretion below 1 mg. daily. Reproducibility of values below 2 mg. per day is poor and the findings may vary as much as 50%. The 24-hour excretion of N^1-Me and of pyridone following an oral test dose of 50 mg. of niacinamide was measured in some of the subjects at the beginning and end of the period of dietary restriction in order to evaluate changes in body stores of niacin.

All urines were analyzed for total nitrogen using the Kjeldahl procedure. Creatinine analyses using alkaline picrate reagent were performed to check completeness of urine collection. Stools were collected for five day periods on several occasions during the course of some of the experiments and fecal nitrogen was determined on aliquots of the homogenates.

Other laboratory tests which were carried out at intervals include the following: gastric analysis, complete blood count, concentration of urea nitrogen in blood and of proteins in serum, glucose tolerance test, basal metabolic rate and liver function tests.[2]

Two experimental diets were formulated to provide niacin in an amount comparable to that reported in some pellagragenic diets (1, 2) and tryptophan in a quantity sufficient to fulfill the minimal requirement for maintenance of nitrogen equilibrium (6). One diet which contained 89 gm. of unenriched corn products was designated the "corn" diet (Table I). The other which contained unenriched wheat flour and farina in place of the corn products was designated the "wheat" diet. The energy value of the basal diets was adjusted to individual need by modifying the sugar and oleomargarine content and varied from 1,700 to 2,000 calories.

In general, each of the subjects consumed all of the food presented each day, except subject 4 who developed anorexia when signs of pellagra appeared. The analysis of the "corn" diet on three separate occasions gave values of 4.7, 4.7, and 4.3 mg. of niacin, 187, 194 and 178 mg. of tryptophan, and 7.0, 7.1 and 6.9 gm. of nitrogen. The "wheat" diet was found to contain 5.7 mg. of niacin, 230 mg. of tryptophan and 7.0 gm. of nitrogen. The content of other nutrients in these diets was calculated to be as follows: vitamin A 11,000 I.U., thiamine 0.5 mg., riboflavin 0.5 mg., ascorbic acid 120 mg., calcium 0.2 gm. and iron 7 mg. The following vitamins[3] were added to the diet of each subject to avoid complicating deficiencies: thiamine 1 mg., riboflavin 1.5 mg., pantothenic acid 5 mg. and pyridoxine 5 mg. Half of the supplement was given with breakfast and half with the noon meal. Two subjects (Nos. 6 and 7) received, in addition, folic acid 2.5 mg., and vitamin B_{12} 5 μg., daily. Subject 7 received a supplement of 2 mg. of niacinamide daily, 0.7 mg. with each meal.

The three subjects (Nos. 4, 5 and 6) who developed signs of pellagra were treated with either niacinamide or tryptophan,[3] and rehabilitation was followed.

RESULTS

In a preliminary experiment, subjects 1 and 2 were maintained on the "corn" diet for 40 and 42

TABLE II

Urinary excretion of N^1-Me after various lengths of time on the "corn" or "wheat" diet

Subject	Diet	N^1-Me excretion, *mg. per day*				
		Days				
		2–13	14–25	26–41	42–61	62–95
1*	Corn	1.8	1.6	0.9	—	—
2†	Corn	2.1	1.4	1.2	—	—
3‡	Wheat	1.9	1.5	1.4	1.3	1.1

* Subject 1 (V. S.) female, age 43, weight 66 Kg.
† Subject 2 (L. M.) female, age 49, weight 65 Kg.
‡ Subject 3 (R. L.) female, age 25, weight 39.5 Kg.

[2] Liver function tests included thymol, zinc and lipid turbidity, cephalin and thymol flocculation, level of serum bilirubin, and bromsulphalein excretion. These tests were performed through the courtesy of Drs. Roy H. Turner, J. Robert Snavely, and William H. Goldwater of the Department of Medicine.

[3] The vitamin and tryptophan supplements used in this study were generously furnished by Winthrop-Stearns Chemical Company, Inc., through the courtesy of Mr. Kenneth Smoot.

TABLE III

Clinical findings in three subjects maintained on the "corn" diet low in niacin and tryptophan

Days* after institution of diet	Subject 4—(L. M.) Age 49, Wgt. 65 Kg.	Subject 5—(H. K.) Age 31, Wgt. 54.5 Kg.	Subject 6—(R. C.) Age 54, Wgt. 44.5 Kg.
50–59	Heartburn, weakness. Atrophy tongue papillae.	Cheilosis (transient). Menstruation absent.	Dermatitis (perirectal).
60–69	Decreased activity, apathy. White patch under tongue. Dermatitis (vulval, perirectal).	Atrophy tongue papillae. Elbows rough.	Red tongue papillae. Heartburn. Diarrhea (one day).
70–79	Weakness, confined to bed. Mouth sore, unable to use false teeth, anorexia, indigestion. Cheilosis, angular stomatitis.	Palpitation. Fissures of tongue.	Numerous vague complaints.
80–89	Depression, dysphagia. "Raw" sensation mouth to rectum. Severe glossitis. Erythema, swelling of oral, vaginal, rectal mucosa. Diarrhea, small ulcers vulva. Bleeding (nose, intestine). Niacin ℞ 82nd day.	Cheilosis (transient). Red tongue papillae.	Atrophy tongue papillae at margin.
90–99		Tongue painful. Skin dry. Angles mouth scaling. Herpes of lip.	Diarrhea (one day). Small ulcer of tongue.
100–109		Depression, hot flushes.	Diarrhea (intermittent). Mouth sore, erythema under tongue.
110–119		Tongue papillae swollen. Lesion left angle mouth. Skin thick and pigmented over finger joints. Skin of elbows thick, dry scaling.	Anorexia, heartburn, cramping abdominal pain, nausea. Erythema oral mucosa, white patches under tongue, glossitis. Diarrhea. Skin thick, pigmented, scaling over terminal phalanges fingers, between thumb and 1st finger. Redness paronychial skin. Niacin ℞ 118th day.
120–129		Skin red, pigmented between thumb and 1st finger (typical pellagrous lesion). Palms and soles dry, scaling. Angular stomatitis.	
130–139		Dermatitis nose, chin, nasolabial folds, perineum. Herpes, cheilosis. Niacin ℞ 135th day.	

* The corn diet was begun on September 10, 1950 in subject 4, on September 12, 1950 in subject 5 and on January 18, 1950 in subject 6.

days and subject 3 on the "wheat" diet for 95 days. No symptoms or signs of nutritional deficiency developed. Urinary excretion of nitrogen remained constant in each subject, mean values being 6.1, 5.8 and 5.2 gm. daily, respectively. In the two subjects who received the "corn" diet, the excretion of N^1-Me decreased to mean levels of 0.9 and 1.2 mg. daily in the last two weeks of the experimental period (Table II). The excretion of pyridone fell to less than 2 mg. within the first two weeks of study and after this time no pyridone could be detected. Excretion of tryptophan averaged 5 to 6 mg. per day.

In subject 3, who received the "wheat" diet, excretion of N^1-Me fell more slowly than in the subjects who received the "corn" diet (Table II).

The decrease in pyridone was approximately the same as that found on the "corn" diet. Mean tryptophan excretion was 11 mg. daily throughout the period of study. The "wheat" diet contained approximately 1.0 mg. more niacin and 40 mg. more tryptophan than the "corn" diet.

Since excretion of niacin metabolites appeared to fall more rapidly on the "corn" than on the "wheat" diet and since no evidence of niacin deficiency was observed after 95 days on the "wheat" diet, long term studies were carried out with the corn regimen. Subjects 4, 5, and 6 were maintained on this diet for 81, 135, and 117 days, respectively, following which niacinamide or tryptophan was administered. Each of the subjects developed signs of niacin deficiency beginning between the 50th and 60th days. Findings included most of the lesions which have been encountered in endemic pellagra (Table III). Anorexia, indigestion and diarrhea occurred in subjects 4 and 6. In subject 4, diarrhea was associated with gross bleeding from the intestinal tract; the mucous membranes became fiery red and swollen and she complained that she felt "raw" from her mouth to her rectum. Atrophy of the tongue papillae occurred in each subject, while in two, glossitis became severe. In one of these, the tongue was bright red and swollen, in the other, moderately red with a clearly delineated area of papillary atrophy on the dorsum. Interesting lesions which occurred in these two subjects were white elevated patches, surrounded by erythema, in the mucous membrane under the tongue and between the gums and cheeks. These lesions could not be scraped off, did not ulcerate and gradually increased in size until treatment was instituted.

Dermatitis of the vulva, perineum or peri-anal region developed in each subject and dermatitis of the elbows in subjects 4 and 5. Typical pellagrous dermatitis of the hands was observed in subjects 5 and 6, in whom niacin deficiency developed relatively slowly. In subject 5, who was maintained on the diet for 135 days, lesions on the hands became red, pigmented, clearly demarcated from normal skin and were symmetrical. This subject also showed dry, thick, slightly pigmented skin over the bridge of the nose and on the chin.

Subjects 4 and 5 developed cheilosis and angular stomatitis and subject 5, dermatitis in the nasolabial folds, lesions previously observed in experimentally induced riboflavin deficiency. All of the subjects were receiving approximately 2.0 mg. of riboflavin daily. An interesting finding in subject 5 was amenorrhea which persisted from the second month on the basal diet until therapy was instituted. Amenorrhea and other disturbances of the menstrual cycle have been noted frequently in endemic pellagra (15, 16).

Depression and apathy were the only mental changes noted. All subjects complained of aching legs and muscle soreness, but these findings could not be considered evidence of nutritional deficiency due to numerous pains which existed prior to initiation of the study. Subject 4 became so weak that she remained in bed during the last few days on the basal diet. No neurological changes were demonstrable in any subject.

Each of the subjects improved rapidly following the addition of niacinamide or tryptophan to the basal diet. Subjects 4 and 5 received niacinamide for 50 and 26 days, respectively, and subject 6 received niacinamide one day (50 mg.) and, after an interval of six days, was given D,L-tryptophan for 26 days. In subjects 4 and 5, who received therapy with niacinamide, subjective improvement was noted within 24 to 48 hours and diarrhea ceased after the first day. Lesions of the lips and oral mucous membrane healed within one week, except for atrophic tongue papillae which required about a month to return to normal. The pellagrous dermatitis showed improvement beginning on the second day, and the skin of the face and hands was practically normal by the 21st day. Approximately one month was required for complete healing of the dermatitis of the elbows. In subject 5, in whom amenorrhea had developed while on the basal diet, menstruation occurred 23 days after niacinamide therapy was instituted. In subject 6, oral lesions improved following the test dose of 50 mg. of niacinamide but did not completely disappear until several days after therapy with tryptophan was instituted. Skin improvement was noted after about two weeks and was essentially complete after the period of tryptophan therapy. At this time regeneration of the lingual papillae was likewise complete.

Urinary excretion of nitrogen and of niacin and tryptophan metabolites was measured in subjects 4, 5, and 6 during the entire course of these

TABLE IV

Urinary excretion findings in subjects on the "corn" diet and following supplements of niacinamide (NAm) or D,L-tryptophan

Subject	Days	Supplement	Nitrogen	Niacin	Quinolinic acid	N¹-Me	Pyridone	Tryptophan
			gm.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
4 (L. M.)	5–21	None	5.2	0.5	2.8	1.2	0–2.5	5
Age 49	22–41	None	5.0	0.4	2.8	0.8	0*	3
Wgt. 65 Kg.	42–61	None	4.9	0.3	2.5	0.7	0*	4
	62–81	None	4.9	0.4	2.0	0.6	0*	4
	82–85	NAm, 10 mg. t.i.d.†	4.7	0.3	2.2	1.2	4.6	3
	86–93	NAm, 10 mg. t.i.d.	4.4	0.3	2.1	1.6	7.0	3
	105–109	NAm, 10 mg. t.i.d.	5.0	0.3	2.2	2.3	11.8	4
	115–126	NAm, 10 mg. t.i.d.	4.5	0.4	2.6	2.9	11.1	3
	128	NAm, 50 mg. test dose				7.6	21.2	
	129–138‡	NAm, 50 mg. t.i.d.	5.1	0.8	2.0	19.6	68.5	4
	142–151§	D,L-tryptophan, 1 gm. t.i.d.	5.0	0.5	7.3	5.9	14.0	40
	154	NAm, 50 mg. test dose				7.9	17.9	
5 (H. K.)	4–19	None	4.6	0.4	4.3	0.9	0.1–1.4	3
Age 31	20–51	None	4.7	0.4	4.2	0.7	0*	3
Wgt. 54.5 Kg.	52–83	None	4.7	0.3	4.5	0.5	0*	3
	84–135	None	4.7	0.3	4.3	0.5	0*	3
	136	NAm, 50 mg. test dose				2.4	14.3	
	137–140	NAm, 10 mg. t.i.d.	5.2	0.4	4.0	1.3	5.6	4
	141–148	NAm, 10 mg. t.i.d.	5.0	0.5	3.9	2.5	9.4	3
	149–152	NAm, 25 mg. t.i.d.	5.2	0.5	3.9	8.0	32.0	3
	153–160	NAm, 25 mg. t.i.d.	5.2	0.5	3.5	7.9	52.0	3
	163	NAm, 50 mg. test dose				7.1	22.1	
	165–174§	D,L-tryptophan, 1 gm. t.i.d.	4.9	0.5	17.8	5.6	18.0	6
	177	NAm, 50 mg. test dose				9.0	27.0	
6 (R. C.)	0	NAm, 50 mg. test dose				5.0	29.0	
Age 54	8–15	None	5.6	0.4	2.3	0.7	0.4–0.7	7
Wgt. 44.5 Kg.	28–31	None	5.8	0.4	2.7	0.5	0*	6
	44–51	None	5.9	0.3	2.5	0.5	0*	6
	64–71	None	5.6	0.3	2.5	0.5	0*	6
	107–114	None	5.1	0.3	2.3	0.5	0*	6
	118	NAm, 50 mg. test dose				3.8	21.3	
	124–127	D,L-tryptophan, 1 gm. t.i.d.	5.4	0.3	7.4	1.0	2.4	40
	128–131	D,L-tryptophan, 1 gm. t.i.d.	5.6	0.4	8.9	2.6	11.4	41
	132–143	D,L-tryptophan, 1 gm. t.i.d.	6.0	0.5	7.3	3.9	13.5	48
	144–149	D,L-tryptophan, 2 gm. t.i.d.	5.7	0.5	11.4	9.1	29.3	85
	152	NAm 50 mg. test dose				9.0	30.4	
	154–159	NAm 10 mg. t.i.d.	5.8	0.4	2.9	5.4	18.1	6
	160–177	NAm 10 mg. t.i.d.	5.3	0.4	2.8	5.2	17.1	7
7 (W. H.)	0	NAm, 50 mg. test dose				7.8	18.2	
Age 36	2–21	NAm, 0.67 mg. t.i.d.	5.7	0.6	4.0	1.4	0.1–1.2	8
Wgt. 68 Kg.	22–61	NAm, 0.67 mg. t.i.d.	5.5	0.5	3.8	1.0	0*	6
	62–89	NAm, 0.67 mg. t.i.d.	5.3	0.5	3.5	0.9	0*	6
	90–121	NAm, 0.67 mg. t.i.d.	5.0	0.5	4.0	0.9	0*	5
	123	NAm, 50 mg. test dose				3.9	17.7	
	125–128	NAm, 10 mg. t.i.d.	5.2	0.5	3.5	2.0	9.5	5
	129–136	NAm, 10 mg. t.i.d.	5.8	0.6	4.5	3.3	15.2	6

* Non-detectable amounts.
† T.i.d.—administered 3 times daily with meals.
‡ Excretion during last 4 days.
§ Excretion during last 8 days.

experiments (Table IV). All subjects remained in nitrogen balance on the basal diet. Fecal nitrogen excretion averaged 1 gm. daily in subject 4 and 0.9 gm. daily in subject 5. Total nitrogen excretion per day in stools and urine measured approximately 6 gm. in subject 4, 5.6 gm. in subject 5. Dietary nitrogen was found by analysis to be 7.0 gm. Subject 5 gained eight pounds in weight during an experimental period of 135 days while subject 4 maintained her weight for two months at which time anorexia developed, food intake decreased and she lost three pounds. In subject 6, fecal nitrogen was not determined, but urinary nitrogen excretion averaged 5.6 gm. daily and she gained two pounds in weight.

The excretion of nicotinic acid, tryptophan and

quinolinic acid (a tryptophan metabolite related to niacin) remained relatively constant on the "corn" diet (Table IV). In each of the three subjects, excretion of N^1-Me decreased gradually, and after two months remained at 0.5 to 0.6 mg. daily. Excretion of pyridone fell rapidly and none could be detected after the first two weeks of the restricted diet.

Following administration of niacinamide to subjects 4 and 5 and of tryptophan to subject 6, excretion of pyridone increased more rapidly than that of N^1-Me. In subject 4, maximum excretion of N^1-Me and pyridone, following a daily dose of 30 mg. of niacinamide, was not attained until this regimen had been continued for approximately three weeks. In contrast to this, maximum excretion of niacin metabolites was reached in five days in a normal subject who had been receiving a good diet and who was given the same amount of niacinamide. The slow rise in excretion of niacin metabolites following administration of niacinamide in subject 4 suggests that body stores of niacin had been considerably depleted. After niacin stores were replenished by therapy with niacinamide, subjects 4 and 5 received 3 gm. of D,L-tryptophan per day, and with this supplement, maximum excretion of niacin metabolites was observed by the third day. In contrast, subject 6 received the same amount of tryptophan after the basal period, and the excretion of N^1-Me and pyridone continued to increase for more than eight days. Therapy with tryptophan for 26 days appeared to fully replenish the niacin stores of subject 6, as evidenced by the very rapid rise in excretion of metabolites following subsequent niacinamide administration. The depletion of body niacin stores by the corn regimen is best shown by the test dose studies in subjects 5 and 6 (Table IV). In subject 5, the excretion of niacin metabolites, following a 50 mg. test dose of niacinamide, was 16.7 mg. after 135 days on the basal diet as compared to 29.2 mg. after therapy with niacinamide (day 163) and 36.0 mg. after tryptophan administration (day 177). In subject 6, excretion of niacin metabolites following the test dose was 34.0 mg. before the basal diet was instituted, 25.1 mg. after 117 days of the restricted regimen and 39.4 mg. after therapy with tryptophan (day 152).

Subject 7 was maintained on the "corn" diet supplemented with 2 mg. of niacinamide daily, one-third of this amount with each meal.[4] No definite signs of niacin deficiency were observed in a period of 122 days. The patient complained of weakness and dizziness during the last month of study but, in view of the severe psychoneurosis which was present, these symptoms could not be attributed to dietary deficiency. Excretion of N^1-Me decreased to 0.9 mg. per day at the end of two months, and remained at this level during the subsequent period on this diet (Table IV). Only traces of pyridone were detected in the urine after the first two weeks. A comparison of the test dose findings after 122 days on the diet with those obtained before this regimen was started (day 0) suggests a small decrease in body stores of niacin. Following administration of 10 mg. of niacinamide three times daily, excretion of N^1-Me and pyridone increased a little more rapidly than in subjects 4 and 5 who had received the "corn" diet alone for 81 to 135 days.

Creatinine excretion showed a slight downward trend in subjects 5 and 7 during the period of study while no changes were observed in subjects 4 and 6. Of the other laboratory tests which were conducted in all subjects, no significant change occurred in basal metabolic rate, level of serum proteins or blood urea nitrogen, glucose tolerance or liver function tests. Some impairment of liver function was evident in subject 5 at the beginning of the study but this was not increased during the experimental period. Electrocardiograms were taken at intervals in subjects 6 and 7 and remained normal.

In subject 4, no free hydrochloric acid was found in the gastric juice after either a test meal or histamine stimulation two months after institution of the "corn" diet. Following therapy with niacinamide, free acid was present in the gastric contents. In subject 5, a slight reduction in gastric acidity was noted during the corn regimen while no change was observed in subject 6 or 7. Complete hemograms were obtained at weekly intervals in all subjects and changes were observed only in subject 4. During the period on the "corn" diet mean values decreased as follows:

[4] The "corn" diet was begun on March 13, 1951.

erythrocyte count from 4.5 to 4.3 million per cmm., hemoglobin from 12.5 to 10.8 gm. per 100 ml., hematocrit from 41 to 37 volumes per cent. No improvement followed administration of niacin or tryptophan for 70 days at the end of which time hemoglobin was found to be 10 gm. per 100 ml. and the erythrocyte count, 4.1 million per cmm. Improvement followed administration of 5 mg. of folic acid three times daily for 12 days and at the end of four weeks the following values were obtained: hemoglobin 11.8 gm. per 100 ml., hematocrit 40 volumes per cent and erythrocyte count 4.4 million per cmm. Although these changes are small, it seems likely that a mild folic acid deficiency may have been responsible for the anemia encountered in this subject. For this reason the diets of subjects 6 and 7 were supplemented with folic acid.

DISCUSSION

The "corn" and "wheat" diets used in the present studies of niacin requirement in man were designed to provide low levels of niacin intake and very little tryptophan in excess of that required for maintenance of nitrogen balance. The recent demonstration that some dietary tryptophan is converted to niacin compounds explains some of the difficulties encountered in previous studies of niacin requirement (1, 2). It seems pertinent to note that administration of tryptophan leads to an increase in the excretion of niacin metabolites both in normal subjects and in patients with pellagra and brings about healing of the lesions of pellagra (3–5, 17–19).

In three subjects maintained on the "corn" diet containing 4.7 mg. niacin and 190 mg. tryptophan, supplemented with other B vitamins, clinical evidence of niacin deficiency first appeared in 50 to 60 days. In one subject who received 2 mg. of niacinamide daily in addition to the same regimen, no signs of niacin deficiency were observed after 122 days nor were any changes noted in a subject maintained on the "wheat" diet, which contained 5.7 mg. niacin and 230 mg. tryptophan, for 95 days.

The excretion of N^1-Me persisted at a low level, 0.5 mg. per day, in the subjects on the "corn" diet during the period in which signs of pellagra were evident. Slightly higher excretion of N^1-Me, 0.9 mg. and 1.1 mg. per day, was found after comparable periods of dietary restriction in subjects on the niacinamide supplemented diet and "wheat" diet, respectively. The addition of 2 mg. of niacinamide per day to the "corn" diet, or the increase of 1.0 mg. in niacin and 40 mg. in tryptophan intake which was provided by the "wheat" diet, appeared to prevent the development of niacin deficiency during the period studied. These data suggest that on the "corn" diet, which furnished about 190 mg. of tryptophan, the minimal niacin requirement is approximately 7 mg. daily. With the "wheat" diet, niacin requirement may be lower because of the larger amount of tryptophan present or because of the difference in the amino acid composition of the protein. The additional 40 mg. of tryptophan in the "wheat" diet, as compared with the "corn" diet, may provide about 1 mg. of niacin compounds (20). The question of whether corn contains a substance which increases niacin requirement remains unanswered. From early times, the association of pellagra with diets high in maize led to the theory that corn contained a toxic substance which was responsible for the disease. More recently, Woolley (21) administered a concentrate of corn to mice and produced a syndrome which could be prevented or cured by administration of niacin. If a "pellagragenic" factor is present in corn, it might increase niacin requirement by interfering with the utilization of niacin or by inhibiting the conversion of tryptophan to niacin. Studies are in progress in persons maintained on "wheat" diets containing the same levels of niacin and tryptophan as were present in the "corn" diet used in the present study. Findings may assist in solving this problem.

Frazier and Friedemann (2) concluded, from their study of records of pellagragenic and nonpellagragenic diets, that "the minimum daily intake of nicotinic acid in a marginal diet containing corn products is about 7.5 mg. per day" and that the niacin requirement may be decreased by improving the quality of the protein in the diet. Data in the present study are in accord with these findings.

Briggs, Singal and Sydenstricker (22) failed to observe an increase in the severity of pellagrous lesions in two subjects with niacin deficiency who

were maintained for nine to 48 weeks on "corn" diets which, according to present calculations, probably contained 3.6 to 4.2 mg. of niacin and 200 to 235 mg. of tryptophan. The diet used by these workers was not supplemented with any vitamins and contained less corn and less protein than did the "corn" diet employed in the present study.

Endemic pellagra usually occurs in persons eating diets deficient in many nutrients and some of the lesions encountered have been attributed to deficiency of factors other than niacin. In the present study of niacin requirement, diets were supplemented with other B vitamins and the lesions which developed can be attributed primarily to niacin deficiency. Cheilosis, angular stomatitis and dermatitis of the nasolabial folds, which have been produced in experimental riboflavin deficiency (23, 24) and by the administration of desoxypyridoxine (25), were also observed in the present investigation. The occurrence of these lesions in pellagra has previously been attributed to concomitant riboflavin deficiency. Subjects in this study were receiving supplements of 1.5 mg. of riboflavin and 5 mg. of pyridoxine daily. The requirements for riboflavin or other B vitamins may be increased when the diet is deficient in niacin. This possibility will be explored in future experiments.

The signs of niacin deficiency which developed during the basal corn regimen differed in the three subjects. Mucous membrane lesions predominated in the subject who was affected relatively early, skin lesions in the two subjects in whom deficiency developed later. The severity of glossitis in the three subjects was related to the rate at which deficiency developed. These data corroborate the clinical impression that different lesions may be observed in acute and chronic deficiency states. The occurrence of mucous membrane lesions without dermatitis in one subject substantiates the validity of a diagnosis of niacin deficiency in the absence of skin lesions, *i.e.*, "pellagra sine pellagra," described in the older literature (26).

Many factors may be responsible for the variations in physical signs of nutrient deficiency observed in subjects receiving apparently similar diets under comparable environmental conditions (27, 28). Individual nutrient requirements may vary as a result of inherent metabolic pattern, body size, total energy needs, caloric intake, and the body stores of a particular nutrient as well as of all other essential nutrients. The relative quantities of other essential substances in the diet, as well as in the tissues, may play a role in the development of physical signs, in that a deficiency of one nutrient may affect the requirement or utilization of others.

The type and location of lesions may be explained, in part, by tissue susceptibility with local trauma as a precipitating influence (29). The similarity of some of the physical signs appearing in subjects receiving diets deficient in either niacin or riboflavin tends to show a common basis for these signs. It is possible that trauma or the susceptibility of particular tissues in different individuals determines which symptoms appear when any one of several essential nutrients is supplied in insufficient amounts.

Gradual depletion of body stores of a nutrient may lead to a different clinical picture than acute depletion as illustrated by the difference in findings between acute and chronic pellagra. Theoretically, time may permit some type of metabolic adjustment to diminishing nutrient supply. If a nutrient participates in several metabolic processes, requirement may vary for each function with certain pathways having priority. Seasonal changes in metabolism have been suggested as a possible explanation of the seasonal occurrence of pellagra (30). The role of each of the above factors and many others is largely speculative and much more information must be forthcoming before definitive statements can be made.

SUMMARY

"Corn" and "wheat" diets low in niacin and tryptophan and supplemented with other B vitamins were designed to study niacin requirement in man. Seven subjects who were maintained on these diets for 40 to 135 days were examined frequently for signs of nutritional deficiency, and the urinary excretion of niacin and tryptophan metabolites was determined. The three subjects who received the "corn" diet, which contained about

4.7 mg. of niacin and 190 mg. of tryptophan, for more than 50 days developed clinical signs of pellagra. No clinical evidence of niacin deficiency was observed in two subjects who received the "corn" diet for 42 days, in one subject who received the same diet supplemented with 2 mg. of niacinamide daily for 122 days, nor in the subject who received the "wheat" diet containing about 5.7 mg. of niacin and 230 mg. of tryptophan for 95 days. All subjects remained in nitrogen balance.

In the subjects who developed pellagra, the excretion of N^1-methylnicotinamide (N^1-Me) decreased to 0.5 to 0.6 mg. per day by the 50th to 60th day (at which time the first signs of niacin deficiency appeared) and remained at that level. In the subject who received the "corn" diet supplemented with 2 mg. of niacinamide daily, excretion of N^1-Me fell to 0.9 mg. per day in 60 days and showed no further decrease. In the subject who received the "wheat" diet, N^1-Me excretion was 1.1 mg. at the end of the experiment on the 95th day. In all subjects, excretion of the 6-pyridone of N^1-Me (pyridone) fell to non-detectable levels after the first two weeks. Excretion of niacin, quinolinic acid, and tryptophan remained essentially constant throughout the experimental period in each subject.

In the subjects who developed pellagra, the low excretion of niacin metabolites following administration of a 50 mg. test dose of niacinamide at the end of the basal period, and the slow increase in excretion of niacin metabolites following therapy with niacinamide or tryptophan, indicated depletion of body stores of niacin.

Although the "corn" diet was supplemented with riboflavin, the clinical signs of niacin deficiency which developed in subjects in this study included some pellagrous lesions which have previously been attributed to concomitant riboflavin deficiency. All of the lesions which developed in these subjects healed rapidly when the "corn" diet was supplemented with niacinamide or tryptophan.

The findings obtained in this study suggest that with a "corn" diet which provides about 190 mg. of tryptophan, the minimal niacin requirement of man is approximately 7 mg. per day.

APPENDIX A

"Wheat" diet low in niacin and tryptophan

Breakfast	gm.	Supper	gm.
Apple juice	100	Green beans*	125
Farina	20 (dry weight)	White bread	30
White bread	40	Oleomargarine	17
Oleomargarine	20	Grapejuice	100
		Sugar	10
		Fruit cocktail or	150
		Apple sauce*	150
Dinner		**Between Meals**	
Mor beef	20	3 p.m.	
Rice	42 (dry weight)	Cookies	40
Beets or	100	Lemon juice	30
Carrots*	125	Sugar	20
White bread	30		
Oleomargarine	20	8 p.m.	
Apple juice	200	Prune juice	100
		Gelatin	20
		Sugar	20
		Pears or	100
		Pineapple*	100

* Canned, drained weight, given alternately.
White flour (Gold Medal) and farina were unenriched.
Analysis of diet: nitrogen 7.0 gm., niacin 5.7 mg., tryptophan 230 mg.
Energy value: approximately 1,900 calories.

APPENDIX B

Recipes used in "corn" and "wheat" diets

White Bread—1 loaf

Unenriched flour	350 gm.
Salt	7 gm.
Sugar	10.5 gm.
Yeast	10.5 gm.
Fat (hydrogenated vegetable)	6.5 gm.
Water	200 gm.

Cooked weight, 520–550 gm.
Baked 15 min. at 425° F.
then 35 min. at 350° F.

Cookies

Unenriched flour	100 gm.
Sugar	68 gm.
Oleomargarine	30 gm.
Baking powder	8 gm.
Salt	2 gm.
Water	48 gm.

Cooked weight, 210–220 gm.
Baked 15 minutes at 500° F.

Biscuits

Unenriched flour	100 gm.
Baking powder	8 gm.
Salt	2 gm.
Fat (hydrogenated vegetable)	12 gm.
Water	80 gm.

Cooked weight, 170–180 gm.
Baked 15 min. at 500° F.

Corn Bread

Cornmeal, degerminated	100 gm.
Unenriched flour	33 gm.
Fat (hydrogenated vegetable)	15 gm.
Sugar	3.5 gm.
Salt	1.5 gm.
Baking powder	8 gm.
Water	130 gm.

Cooked weight, 250–260 gm.
Baked 30 min. at 500° F.

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IRON METABOLISM. IRON STORES IN MAN AS MEASURED BY PHLEBOTOMY [1]

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Hemosiderin and ferritin represent storage iron, available for hemoglobin synthesis when need arises. Previous observations (1, 2) have indicated that these stores must be depleted before iron deficiency becomes manifest in the circulating red cell mass. The depletion of iron stores associated with normal hematological measurements may therefore be regarded as the "preclinical" phase of iron deficiency.

Interest in the pathogenesis of iron deficiency anemia has led us to further methods of evaluating iron stores. A rough estimate of iron reserves may be obtained by examination of sternal marrow for hemosiderin (2). A more quantitative measurement may be made by determination of iron mobilized for red cell production following phlebotomy. Data obtained by the latter technique are presented to define the size of the iron storage compartment in order to determine its availability and rate of replenishment in normal adult males and in subjects with abnormalities of iron metabolism.

METHODS

Volunteers were repeatedly phlebotomized as previously described (3). A balance sheet was kept on each subject recording the amount of iron removed by phlebotomy and the weekly change in red cell mass expressed in mg. of iron. Repeated blood volumes were performed in each subject, in some individuals weekly over a period of two months. Despite the development of anemia the total blood volume showed no change thought to be significant. The current RBC mass was calculated by multiplying the average blood volume by the hematocrit.

The iron balance was determined for each weekly period. All figures were expressed in terms of mg. iron, using a value of 1.1 mg. iron/1.0 ml. packed red cells. The calculations [5] involved the following steps:

1). ml. packed red cells removed $\times$ 1.1 = total mg. iron removed.

2). (ml. red cell mass at beginning of week minus ml. red cell mass at end of week) $\times$ 1.1 = mg. iron deficit in circulating red cell mass.

3). (*1*) $-$ (*2*) = mg. iron mobilized for hemoglobin synthesis. The figure represents "available" iron.

During the experimental period an unknown amount of iron was absorbed. Previous studies indicated that 3 mg./day represents maximal iron absorption from a normal diet (3). This correction was made in converting "available" to storage iron (Table I).

Blood for hematocrit determination was centrifuged in Wintrobe Tubes for one hour at 3,000 r.p.m. (centrifuge radius 6 inches). Blood volumes were determined by the method of Gibson and Evelyn, with a correction factor of 0.85 (4). Serum iron was determined by the method of Kitzes, Elvehjem, and Schuette (5).

EXPERIMENTAL PROCEDURE

Subjects were phlebotomized approximately 500 ml. weekly for seven weeks. In those instances where iron stores were limited, the degree of anemia produced necessitated cessation of the bleedings before the seventh week. All but one subject were on normal diets with estimated iron content between 10 and 20 mg./day. For descriptive purposes our subjects have been divided into six categories.

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[5] Sample calculation:

Basal blood volume 5,000 ml.

Week 4—hematocrit 40.0%; erythrocyte mass 2,000 ml.

Week 5—hematocrit 38.4%; erythrocyte mass 1,920 ml.

200 ml. erythrocytes removed by phlebotomy at week 4.

1). 200 $\times$ 1.1 = 220 mg. iron removed.

2). (2,000–1,920) $\times$ 1.1 = 88 mg. iron deficit in erythrocyte mass.

3). 220 $-$ 88 = 132 mg. "available" iron.

TABLE I

Storage iron mobilized in seven weeks

	Iron removed	Available iron*	Per cent of iron removed replaced by storage iron
	mg.		
I. Normal	1,704	844–991	50–58%
II. Prefed with iron	1,626	890–1,037	55–64%
III. Iron depleted	1,150	93–198	8–17%
IV. Oral iron	1,810	1,294–1,441	71–80%
V. Enlarged iron reserves	1,518	920–1,067	61–70%
VI. Hemochromatosis	1,601	1,179–1,326	71–83%

* The first figure represents allowance for maximal absorption of dietary iron (3 mg./day). The second figure is without allowance for absorption. True estimate of storage iron is probably between these two figures.

I. *Normal iron stores.* Two men, ages 25 and 30 years, in good health had no history of previous blood loss or iron administration. Blood values were normal.[6]

II. *Normal iron stores enhanced by oral iron.* Two men, ages between 25 and 30 years, had taken iron by mouth (200 and 400 gm. $FeSO_4$) over a six-month period up to one year before the start of this study. They also had normal hematological measurements.

III. *Iron depleted.* Two men, ages 26 and 33, had normal hematological measurements. Both had given numerous blood transfusions but none during the two months immediately preceding this study.

[6] Reference to normal blood values indicates the following: red count 4.5 to 5.5 × 10^6/cu.mm., hemoglobin 14.5 to 16.5 gm./100 ml., hematocrit 43–50%, mean corpuscular volume 80–95 cu.μ, mean corpuscular hemoglobin concentration 31–36%, serum iron 90–150 μgm./100 ml.

IV. *Supplementary oral iron during the phlebotomy period.* Two young men were given 0.6 and 1.2 gm. $FeSO_4$ per day during the period of bleeding. Since no difference was observed in the response to the two doses of iron, they were grouped together. Hematological measurements were initially within normal limits.

V. *Increased iron stores.* Two men, ages 52 and 54, were brothers of patients with hemochromatosis. These subjects had no clinical evidence of the disease but had elevated serum iron values and an increase in sternal marrow hemosiderin.

VI. *Hemochromatosis.* Four patients, three male and one female, all with the diagnosis of hemochromatosis established by liver biopsy, had elevated serum iron and increased marrow hemosiderin.

Other hematological data in these subjects including hematocrit changes, serum iron values, and calculated rates of hematopoiesis may be found in a previous report (3).

RESULTS

The weekly mobilization of iron is portrayed in cumulative line graphs. In all experimental subjects there was a lag period of one week after the initial phlebotomy before iron stores were utilized for blood production.

Normal subjects (I and II) after seven phlebotomies showed evidence of iron depletion. Hematocrits had fallen to 33%, and the rate of hematopoiesis was curtailed. Over these seven weeks 50 to 60% of the iron removed had been replaced from iron stores (Figure 1).

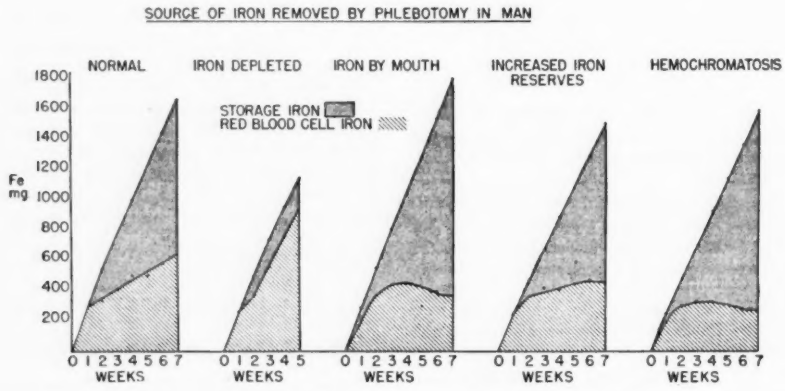

FIG. 1. THE CUMULATIVE IRON BALANCE OVER A SEVEN-WEEK PHLEBOTOMY PERIOD

In each group, the sloping left-hand line indicates total iron removed by weekly 500 ml. phlebotomies. The lower, cross-hatched area represents red cell iron deficit, calculated from the degree of anemia, and the remaining upper area represents iron mobilized from the tissue stores. The available storage iron varies directly with the size of the body stores. Oral supplementary iron is as available as tissue stores, if given during the period of bleeding.

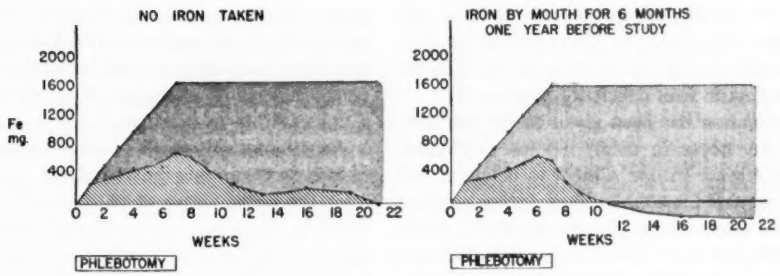

FIG. 2.

The "normal" group is here divided into two subjects who had taken no supplementary iron prior to the phlebotomy period (group I), and two subjects who had taken large amounts of ferrous sulfate one year before the study (group II). The latter pair showed a more rapid recovery and mobilized about 200 mg. more iron.

In the recovery period (Figure 2) a difference was discernible for the first time between I and II. Those individuals prefed iron overcame the red cell deficit by the 11th week, whereas in the normal subjects a deficit was present until the 21st week. Total storage iron available for hemoglobin production over a 14-week period in group I was 1,200 to 1,500 mg. and in group II, 1,450–1,750 mg.

Previously phlebotomized subjects with normal hemograms at the time of the study (group III) tolerated bleeding poorly (Figure 1). After removal of 2,500 ml. of blood no more than 200 mg. of iron had been mobilized from stores, and the hematocrit had fallen below 30%. To exclude the possibility that lack of iron mobilization might be due to impaired hematopoiesis from other causes, these subjects were fed iron with an excellent hematopoietic response.

To investigate further the effect of previous blood loss on iron stores, subject D. H., one of the normal group, was bled again one year after the initial course of phlebotomies. In the interim he had been on a normal diet without iron supplements. Red cell measurements and serum iron had returned to normal. Four phlebotomies resulted in a more severe anemia and less mobilization of iron than the year before (Figure 3).

In subjects given iron by mouth (group IV), in those with increased iron reserves (V) and in patients with hemochromatosis, from 61 to 83% of the iron removed was replaced by storage iron (Figure 1).

DISCUSSION

Iron stores provide protection against the development of iron deficiency anemia. The direct relationship between the presence of adequate iron stores and the ability to accelerate blood production after blood loss has been previously discussed (3). It is our further conviction that if hemo-

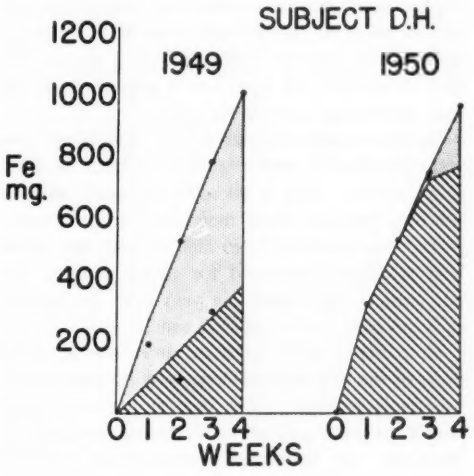

FIG. 3.

Subject D. H. of group I was followed to recovery of blood and serum iron from the first seven-week phlebotomy period, and was subjected to similar bleedings one year later. There was an evident lack of available storage iron during the second study. The exhausted stores had not been repleted during one year on a normal diet, despite the prompt restoration of normal hemoglobin levels.

siderin and ferritin are present in tissues, this storage iron is available for blood production (1). This is borne out by the absence of hemosiderin in the marrow in iron deficiency anemia (6).

Little attention has been given to the quantitation of iron stores in man. Hynes (7) calculated iron reserve in one subject to be about 600 mg. after repeated small bleedings during a period of over 100 days. Hahn (8), by chemical analysis of iron deficient and normal dogs, has estimated storage iron to be 20% of total body iron. Translated to man with approximately 4.5 gm. of body iron, this would represent 900 mg. of storage iron.

Our studies were designed to minimize effects of absorption by removing large amounts of iron over a short period of time. Since previous observations indicate that a normal diet contributes a maximum of about 3 mg. iron for hemoglobin production per day (3), correction for diet will change our figures of storage iron relatively little (Table I). It is also assumed that the "pull" on iron stores is probably directly related to the degree of anemia: the greater the anemia, the greater the rate of iron mobilization (9). This would tend, if anything, to reduce the differences observed between the groups, since those patients with smaller stores would develop a greater degree of anemia and therefore a greater "pull" on iron remaining in their tissues.

In normal adults approximately 900 mg. of iron were mobilized in seven weeks and 1,200 to 1,500 in 14 weeks. This is taken to represent most of the iron stores of these individuals since hematopoiesis was retarded from lack of iron, the serum iron remained depressed for many months, and one subject bled again one year later had practically no iron reserve at that time.

In patients with increased iron stores, about 80% of the red cell iron removed by phlebotomy was replaced from tissue iron stores over seven weeks. In the patient with hemochromatosis, if bleedings are continued, replacement reached 100% as the initially induced anemia regresses despite the continued weekly phlebotomies.

Of particular clinical interest is the individual with decreased iron stores. It is to be expected in iron deficiency anemia that blood loss will be reflected quantitatively in the circulating red cell mass. However, it is also evident that subjects with a history of previous blood loss (group III) may have completely normal hematological values, yet have depleted stores and show almost as great a vulnerability to iron loss.

An attempt was made to measure the repletion of iron stores in one individual (D. H.) one year after the initial series of phlebotomies. As indicated by Figure 3, a normal diet appears to result in a negligible accumulation of iron over this period of time. In another subject (group III) a series of phlebotomies had been followed by six months of iron ingestion (200 gm. $FeSO_4$). Phlebotomies performed subsequently showed negligible iron stores.

Our observations are similar to those reported by Fontès and Thivolle (10) based on tissue iron analyses. In dogs made anemic by bleeding, oral iron was administered for six weeks, restoring the peripheral blood to normal. At this time tissue iron stores were non-existent. Similarly in anemic dogs on supplementary iron for six to 12 months, iron reserves were found to be only one-third to one-half of normal. It would appear that orally administered iron is absorbed in increased amounts until anemia is corrected, but that iron stores are not easily reconstituted by the oral route. One is impressed that iron stores are accrued gradually over many years, and when depleted are rebuilt slowly.

There is considerable question also whether orally administered iron is effective in increasing iron stores above normal, in contrast to its efficacy in the treatment of anemia. In animals on a normal diet it is difficult to increase body iron unless very large amounts of iron are administered (1, 11). Similarly, in this study the prefeeding of iron (group II) had little effect on the amount of available iron. Iron feeding for six months increased iron stores by about 200 mg.

The availability of iron from the diet and from stores may be compared on the basis of the data presented. Three separate situations should be distinguished—*1*) adequate iron stores, *2*) medicinal iron by mouth, *3*) normal diet with iron content of approximately 15 mg./day. In subjects with abundant storage iron 30 to 40 mg. of iron were available daily for hemoglobin production.

Medicinal iron given orally supplies comparable amounts of iron for hemoglobin production. However, previous studies indicate that an average of only 3 mg. of dietary iron is available in the iron-deficient subject. Since the normal bone marrow uptake daily for hemoglobin synthesis is about 25 mg., stores may allow an increase of up to 150% above normal, while dietary iron allows an increase of 15%. Thus the individual with adequate iron stores is ten times as effective in accelerating hematopoiesis following blood loss as the individual with depleted stores who must derive iron from his diet.

SUMMARY

Available iron stores in man may be measured by the mobilization of tissue iron in response to repeated phlebotomies. Retarded hematopoiesis toward the end of the bleeding period is assumed to indicate near depletion of available iron.

The normal adult male had iron stores of 1,200 to 1,500 mg., mobilized over a period of three to four months.

Prolonged iron feeding prior to the phlebotomies had little effect on the size of stores in the normal individual.

Once iron stores had been depleted, they were rebuilt slowly, either from a normal diet or from orally ingested supplementary iron.

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ABNORMALITIES IN N^{15} EXCRETION RATES AFTER INGESTION OF TAGGED GLYCINE IN CUSHING'S SYNDROME AND FOLLOWING ACTH ADMINISTRATION [1]

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The purpose of this paper is to report studies of excretion rates of isotopic nitrogen, N^{15}, following the ingestion of glycine in which the isotope was chemically incorporated. The studies were carried out on three patients with untreated Cushing's syndrome, and on two of these patients after receiving therapy in the form of X-ray to the pituitary and testosterone propionate respectively. The control studies on healthy volunteers and studies on two of these volunteers after receiving ACTH on three occasions are also reported.

MATERIALS AND METHODS

All subjects were maintained on a constant diet containing approximately 1 gm. of protein per kilogram of body weight. The nitrogen balance was estimated by analysis of an aliquot of a 24 hour urine specimen by the macro Kjeldahl technique (1). An analysis of an aliquot of the diet was made at least once for each study. The stool nitrogen was assumed to be 1.3 gm. daily (2). Breakfast contained, in addition to dietary glycine, a measured amount of glycine which varied from 40 to 120 mg./Kg. of body weight in different experiments. On the test day, instead of an addition of the ordinary glycine, the subject received an equivalent amount of additional glycine in which isotopic nitrogen, N^{15}, had been chemically incorporated. The amount of N^{15} ingested varied from 1 to 3 mg./Kg. of body weight. Urine collections were made every six hours for the first 48 hours, every 12 hours for the next 48 hours, and a final 24 hour collection was made on the fifth day.

An aliquot of each urine specimen was prepared for the mass spectrometer by the method of Sprinson and Rittenberg (3), utilizing the macro Kjeldahl technique with mercuric sulfate as a catalyst, digestion being carried out for 12 hours. The ammonia was distilled into hydrochloric acid, the nitrogen being liberated from the ammonium chloride with sodium hypobromite in an appropriate vacuum system.

The N^{15}/N^{14} ratio was then determined by means of the mass spectrometer of the Nier type with a single slit beam with an accuracy of $\pm$ .002% atom excess.

The rate of excretion of ingested N^{15} was determined in the following subjects:

1. Eight studies on seven healthy volunteers—K. C., A. W., A. E., A. W., R. H., J. M., (♂); S. B. (♀).
2. Four studies on two female patients (M. C. and D. M.) with Cushing's syndrome in nitrogen equilibrium.
3. One female patient (E. D.) with Cushing's syndrome in slightly negative nitrogen balance.
4. Patient M. C. with Cushing's syndrome in positive nitrogen balance while receiving 25 mg. of testosterone propionate daily after pretreatment with testosterone propionate 25 mg. daily for five days.
5. Patient E. D. with Cushing's syndrome in slightly positive nitrogen balance five months after having received X-ray therapy to pituitary.
6. Three studies on two healthy volunteers while receiving ACTH. In study No. 1 on A. W., the subject received ACTH 25 mg. every six hours for six doses beginning at the time the N^{15} was administered. In study No. 2 on A. W., the subject received two 25 mg. injections of ACTH every four hours before the N^{15} was given, and 25 mg. every four hours for six doses after the N^{15} was given. ACTH induced negative nitrogen balance each time. Subject A. E. received four 25 mg. injections of ACTH every four hours before the N^{15} was given and 25 mg. every four hours for four doses after the N^{15} was given; nitrogen balance was maintained throughout.

RESULTS

It should be noted that healthy subjects, known to be in nitrogen equilibrium on a diet containing approximately 1 gm. protein per kilogram of body weight, excreted the ingested N^{15} at a remarkably similar rate (Figure 1, Tables I and II). This excretion of 25.9% (S.D. $\pm$ 1.6) in 24 hours compares favorably with the rate of 30% reported by Sprinson and Rittenberg (4). Our studies corroborated Rittenberg's observation that the N^{15} was mainly in the urea fraction. It is of interest that the excretion rates were apparently uninfluenced by the range of tagged glycine ingested from 40 mg./Kg. to 120 mg./Kg. in our hands and 10 mg./Kg. by Sprinson and Rittenberg (4). This constancy of N^{15} excretion on a "normal"

[1] We wish to thank Miss Sarah Brent and Miss Sara Crumley of the Dietary Department, University of Virginia Hospital, for their helpful assistance.

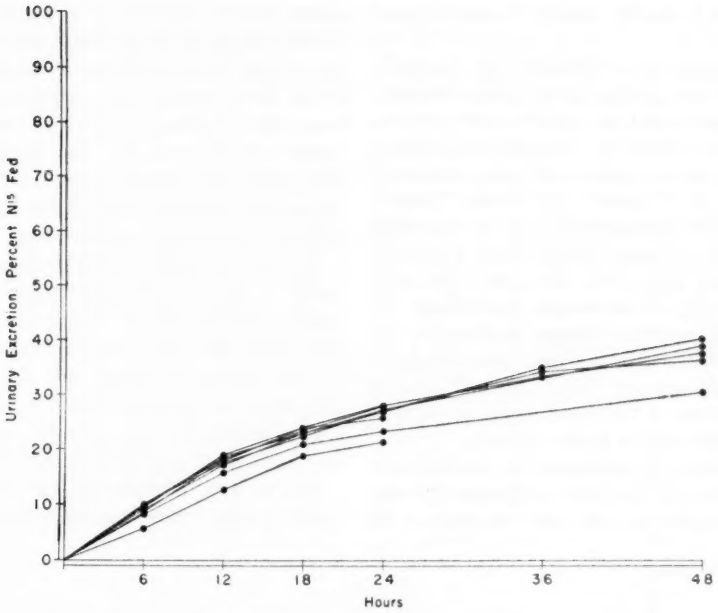

FIG. 1. ACCUMULATIVE N^{15} EXCRETION PLOTTED AS PERCENTAGE OF N^{15} INGESTED IN HEALTHY SUBJECTS IN NITROGEN EQUILIBRIUM ON A STANDARD DIET

TABLE I

Subject	Wt. range (Kg.)	Daily nitrogen balance									
		Days before tag					Days after tag				
		5	4	3	2	1	1	2	3	4	5
Normals											
A. W. 1	95.4–97.7	−1.2	−1.4	−0.2	−0.3	−1.0	−0.2	+0.2	+0.4	+0.7	−1.1
A. W. 2	91.8–93.6	−0.2	−0.1	−0.2	−0.1	−0.1	−0.1	−0.7	−0.5	—	—
R. H. 1	73.6–74.2	0.0	+1.0	+0.9	−0.4	−1.4	+0.7	−1.4	+0.6	−0.4	−0.4
K. C.	77.8–78.3	+0.3	−1.1	+0.8	+0.7	+0.9	+0.6	−0.9	−0.3	—	—
S. B.	51.4–52.2	—	−1.2	−0.6	−0.8	−1.2	−0.4	−0.7	+0.3	−0.9	−1.2
A. E.	76.6–77.8	+0.8	+0.9	−0.5	−0.1	+0.6	+0.6	−0.2	—	—	—
J. M.	80.0–80.1	−3.8	−3.3	−2.1	−1.2	−0.3	−0.6	−0.3	+0.4	+0.1	—
A. W.	82.0–82.5	−1.0	0.0	−1.1	+0.7	−0.2	−0.6	−0.4	−0.2	+2.0	+1.6
Cushing's syndrome											
M. C. 1	62.8–63.6	—	+0.5	−0.1	−0.4	+1.4	+0.1	+0.4	+0.3	+0.3	+0.6
M. C. 2	63.5–63.8	−0.1	−0.2	−0.4	−0.4	−0.3	−0.5	−0.7	+0.6	+0.2	−0.7
M. C. 3	61.3–61.6	0.0	+0.2	−0.4	−0.5	−0.4	−0.5	−0.4	0.0	−0.2	—
M. C. 4*	63.4–65.0	+2.0	+2.3	+2.6	+2.6	+3.7	+3.0	+3.2	+2.6	+2.5	+3.1
E. D. 1	73.3–73.9	−1.7	−1.9	−1.5	−2.2	−1.4	−2.3	−1.4	−1.4	−2.4	−0.3
E. D. 2†	72.4–73.9	+2.3	+1.4	+2.6	+2.3	+1.6	+0.6	+1.6	+1.8	+0.9	+1.9
D. M.	66.7–67.5	+0.9	+1.2	−0.3	−1.2	+0.1	+0.6	+1.8	+0.7	−0.8	+1.2
Normals plus ACTH											
A. W. 1	91.1–93.0	+1.3	+2.2	+1.1	+1.1	+0.6	−1.9	−4.4	−1.3	−2.7	—
A. W. 2	89.9–91.2	—	+0.7	+1.3	+3.5	−1.0	−6.4	−3.4	−2.3	−3.7	−1.6
A. E.	76.5–78.6	+2.0	+0.3	−1.9	+0.5	−1.7	+0.4	+0.8	+1.0	+0.1	—

* Patient received testosterone propionate 25 mg. daily for five days previous to and during study.
† Five months after X-ray therapy to pituitary.

protein intake in healthy subjects is most impressive.

In four studies on two patients with Cushing's syndrome on diets similar to the control subjects, it was established that the patients were in nitrogen equilibrium (Table I). One additional patient was found to be in negative nitrogen balance of *circa* 2.0 gm. in 24 hours. All of these patients showed a striking abnormality in the excretion rates of the N^{15} (Figures 2 and 3, Tables I and II). The rates were 48%, 52%, 47%, and 42% in 24 hours in the subjects in nitrogen equilibrium. In the patient in negative nitrogen balance the excretion rate was 50% in 24 hours. It is worthy of note that approximately 90% of the N^{15} was in the urea fraction of the urine in the subjects in Cushing's syndrome as in the controls.

When testosterone propionate was administered to the patient with Cushing's syndrome who was in nitrogen equilibrium she went into positive nitrogen balance and her N^{15} excretion fell from the high abnormal levels (48% and 51%) toward the normal (32%) (Figure 2, Tables I and II). When X-ray therapy was administered to a patient with Cushing's syndrome resulting in a partial clinical remission, the negative nitrogen balance became slightly positive and the high abnormal N^{15} excretion rate (50%) fell toward normal (38%) (Figure 3, Tables I and II).

On two occasions ACTH administration resulted in negative nitrogen balance in a healthy subject and the N^{15} excretions were abnormally high, 36% and 45%, compared with his baseline of 27% (Figure 4, Tables I and II). ACTH did not disturb the nitrogen balance in another healthy subject but despite this, the N^{15} excretion rose from 27% to 39% (Figure 4, Tables I and II).

This striking alteration in the handling of ingested glycine despite the maintenance of nitrogen

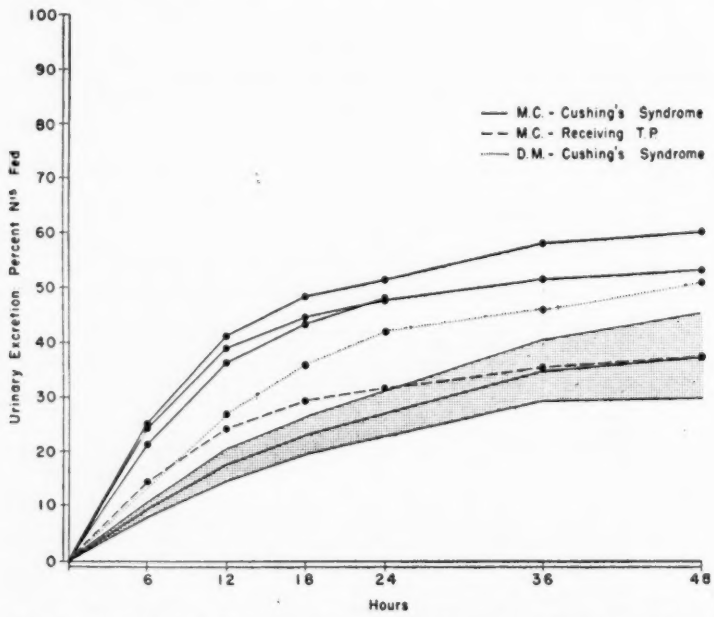

FIG. 2. ACCUMULATIVE N^{15} EXCRETION OF FOUR EXPERIMENTS ON TWO PATIENTS WITH CUSHING'S SYNDROME (M. C., D. M.) IN NITROGEN EQUILIBRIUM ON A STANDARD DIET. ADDITIONAL CURVE ON PATIENT (M. C.) RECEIVING TESTOSTERONE PROPIONATE IN POSITIVE NITROGEN BALANCE

The hatched area portrays the mean excretion rate plus or minus two standard deviations in seven healthy subjects in nitrogen equilibrium on a standard diet.

TABLE II

Subject	Weight	Nitrogen intake		Urinary nitrogen						
		N^{14}	N^{15}	N^{14}	N^{15} accumulated excretion					
		24 hrs.	Single dose	24 hrs.	6 hrs.	12 hrs.	18 hrs.	24 hrs.	48 hrs.	24 hrs.
	(*Kg.*)	(*gm.*)	(*mg.*)	(*gm.*)	(*mg.*)	(*mg.*)	(*mg.*)	(*mg.*)	(*mg.*)	(%)
Normals										
A. W. 1	97.0	16.1	104	14.8	10.3	18.1	23.2	28.0	39.8	27.0
A. W. 2	93.5	16.1	104	13.2	10.5	18.9	24.3	29.3	39.4	28.1
R. H.	74.1	12.9	82	10.9	7.4	14.1	18.9	22.2	33.3	27.0
K. C.	77.9	14.9	33	13.0	3.3	6.1		8.9	12.8	27.0
J. M.	80.0	12.1	33	11.4	2.7	5.2	6.9	7.6	10.2	23.0
A. E.	77.3	14.3	180	12.5	16.2	32.9	43.3	49.9		27.5
A. W.	82.3	16.8	90	16.1	5.7	11.8	16.9	19.7		21.7
S. B.	51.6	8.5	150	7.6	14.6	28.2	35.7	38.6		26.0
Mean										25.9 S.D.±1.6
Cushing's syndrome										
M. C. 1	62.9	10.9	78	9.5	19.3	30.7	35.3	37.6	42.1	48.2
M. C. 2	63.7	10.9	39	10.1	9.9	16.2	18.9	20.2	23.5	51.7
M. C. 3	61.5	10.9	22	10.1	4.6	7.8	9.5	10.4		47.3
M. C. 4*	64.7	10.9	52	6.6	7.5	12.6	15.3	16.6	19.4	31.9
E. D. 1	73.7	14.0	120	15.0	26.3	45.0	54.7	60	71.4	50.0
E. D. 2†	73.8	14.0	180	12.1	30.3	51.9	63.8	69.2		38.3
D. M. 1	67.0	11.8	120	11.1		32.0	43.5	50.7	60.4	42.2
Mean										47.9 S.D.±3.5
Normals plus ACTH										
A. W. 1	91.3	16.1	104	16.4	13.8	25.1	31.8	37.3	50.0	36.0
A. W. 2	90.0	16.0	104	20.9	17.2	31.9	40.1	47.0	60.2	45.3
A. E.	77.6	14.3	33	12.6	5.2	8.8	11.2	13.0	16.5	39.4

* Patient received testosterone propionate, 25 mg. daily for five days previous to and during the study.
† Five months after X-ray therapy to pituitary.

equilibrium parallels the observations noted above in Cushing's syndrome.

DISCUSSION

The classical nitrogen balance study offers little insight concerning alterations in intermediary metabolism of amino acids and protein. Albright (5) has called attention to the common finding of "nitrogen balance" in Cushing's syndrome despite the clinical evidence of a profound disturbance in protein metabolism. A combination of nitrogen balance studies with the ingestion of glycine tagged with N^{15} offers new information in the study of this problem and other problems concerned with disturbances in protein metabolism.

Sprinson and Rittenberg (4, 6) offered a mathematical analysis of the excretion rates of N^{15} in healthy subjects following the ingestion of tagged glycine. With full realization of the assumptions involved, they postulated homogeneity of the "metabolic pool" and calculated its size and also the rate of tissue synthesis. We presented a preliminary report of our data (7) using this type of analysis. In the above analyses the rate of urea excretion was not considered to be a significant factor. Tarver (8) has criticized these assumptions and has pointed out that the size of the metabolic pool appeared to be too large. Rittenberg and San Pietro (9) have reappraised the problem and recalculated the size of the metabolic pool after first determining the size of the urea space. This newly derived pool size is considerably smaller and more probable than the original figures. Unfortunately the data presented here will not permit analysis by this revised technique.

However, even without mathematical treatment of the data we feel that it is of interest to report the striking alterations in the handling of the ingested N^{15} in Cushing's syndrome before and after therapy and in healthy subjects following the administration of ACTH. It seems reasonable to conclude from the data presented that when ni-

trogen equilibrium exists in Cushing's syndrome, and following ACTH administration, the observed alteration from normal in N^{15} excretion indicates that a larger fraction than normal of ingested glycine nitrogen is converted to urea and excreted in 24 hours, and conversely, a smaller fraction is utilized for synthetic or anabolic processes. Inasmuch as the subject is in nitrogen equilibrium, it may be assumed that the rate of tissue breakdown equals the rate of tissue synthesis. When nitrogen balance is altered, as following testosterone propionate, X-ray therapy, and ACTH therapy, speculation may be unwarranted.

If one may generalize from the alteration in the handling of the tagged glycine nitrogen, these studies appear to suggest both decreased protein synthesis and increased degradation of ingested amino acids in hyperadrenocorticism, and offer partial support to Albright's concept of the role of antianabolism in this disorder. On the other hand, Kinsell and associates (10) found no depression of the rate of incorporation of S^{35} into plasma protein following the administration of methionine tagged with S^{35} in Cushing's syndrome.

It is recognized that an alternative interpretation would fit the observations reported here; namely, that hyperadrenocorticism is associated with an increased turnover rate of protein in general; that is, increased synthesis and increased degradation.

However, our interpretation of the data seems more reasonable and is in general accord with isotope studies of protein metabolism and adrenal function in animals.

Friedberg (11) observed that the administration of dehydrocorticosterone to adrenalectomized animals depressed the rate of incorporation of methionine sulfur (S^{35}) into tissue protein.

White, Hoberman, and Szego (12) injected glycine tagged with N^{15} and reported a higher N^{15} content in the tissues of adrenalectomized fasted mice than in the normal fasted animals.

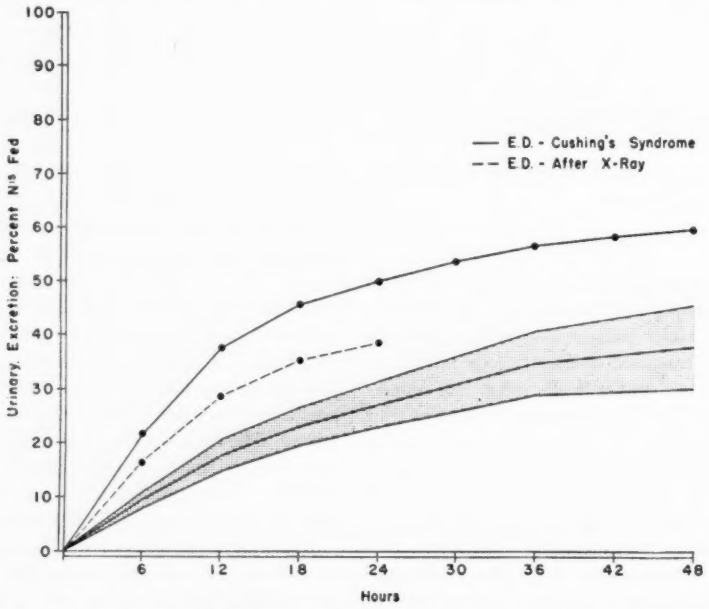

FIG. 3. ACCUMULATIVE N^{15} EXCRETION IN PATIENT (E. D.) WITH CUSHING'S SYNDROME IN TWO EXPERIMENTS

Untreated, the patient was in mild negative nitrogen balance; five months after X-ray treatment, was in mild positive nitrogen balance.

The hatched area is as described in Figure 2.

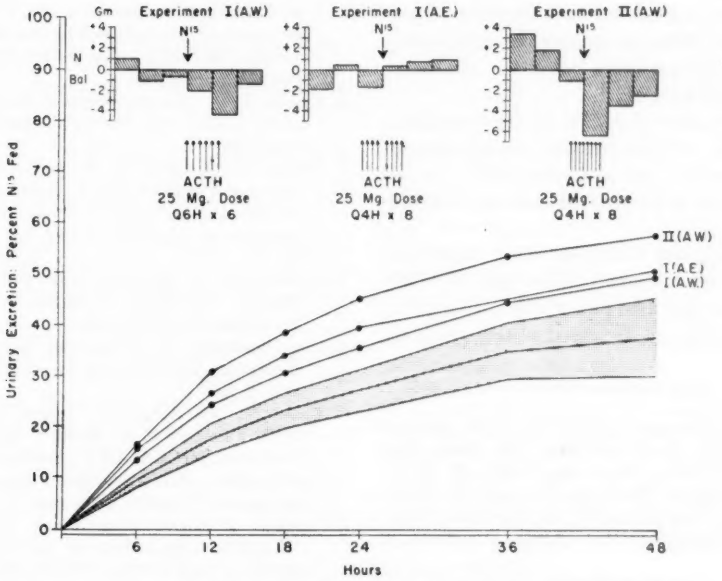

FIG. 4. ACCUMULATIVE N^{15} EXCRETION RATES IN THREE EXPERIMENTS ON TWO HEALTHY SUBJECTS (A. W., A. E.) ON A STANDARD DIET WHILE RECEIVING ACTH

The mode of administration of ACTH and the alterations in nitrogen balance in each experiment are portrayed.

The hatched area is as described in Figure 2.

Clark (13) fed glycine tagged with N^{15} to normal and adrenalectomized rats. He injected cortisone and studied urinary excretion rates. Without publication of the data, he reported, "In the animals receiving cortisone there was a decrease in protein synthesis as compared to the control animals."

Hoberman (14) injected fasted rats with glycine tagged with N^{15} and studied urinary excretion rates after hormonal manipulation of various sorts. It is germane to this paper that among his conclusions he states, "the biochemical reactions leading to the formation of protein are accelerated in adrenalectomized and hypophysectomized rats" and finally, "the synthesis of body proteins is diminished in those animals in which the adrenal cortex is hyperactive." He subjected his data to mathematical treatment somewhat similar to Sprinson and Rittenberg (4), and hence it is open to the same criticism.

Marshall and Friedberg (15) reported that ACTH administration to hypophysectomized rats decreased the uptake of the C^{14} label of carboxyl marked glycine in muscle and kidney protein.

Barton (16) has injected glycine-2-C^{14} into normal and adrenalectomized mice. He has found in either case that prior injection of a small dose of adrenal cortical extract will inhibit the abrupt rise in the specific activity of the uncombined glycine in the liver, and the catabolism of the glycine is increased.

SUMMARY AND CONCLUSIONS

1. The rate of N^{15} excretion in the urine following ingestion of tagged glycine has been determined on healthy subjects in nitrogen equilibrium on a standard diet.

2. In four studies on patients with Cushing's syndrome an increased rate of excretion of N^{15} was noted while the subject was in nitrogen equilibrium. In one patient in negative nitrogen balance, there was a similar degree of alteration in N^{15} excretion.

3. An X-ray induced partial remission in one

case of Cushing's syndrome and testosterone propionate administration in another resulted in positive nitrogen balance and a fall in the N^{15} excretion rate toward normal.

4. Administration of ACTH on three occasions to two healthy subjects resulted in negative nitrogen balance on two occasions only. In all these studies the N^{15} excretion rate was similarly increased.

5. It is suggested that these data support the concept that hyperadrenocorticism may be characterized by an increased rate of degradation of ingested amino acid and decreased protein synthesis.

ACKNOWLEDGMENTS

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THE EFFECTS OF ACETYLCHOLINE AND METHACHOLINE UPON THE HUMAN COLON [1]

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INTRODUCTION

The association of life stress, emotional conflict, and disordered function of the colon has often been observed clinically and has recently been confirmed by experimental observation on man (1, 2). Because of the established importance of the autonomic nervous system in the integration of reactions to stress, it has been assumed that the disturbed activity of the colon is directly caused by autonomic stimulation. Yet little is known about the effects of sympathetic or parasympathetic stimulation on the human colon. Since the musculature of the colon is innervated by post-ganglionic parasympathetic fibers, the effect on the bowel of acetylcholine, the chemical mediator of these impulses, is of considerable interest. Numerous investigators have observed that acetylcholine enhances tone and peristaltic activity of the stomach and intestines in experimental animals both *in vitro* and *in vivo* (3–5). Nothing is known of its effect on coordinated motor activity of the human gastrointestinal tract. We have, therefore, studied its effect on the motility of the intact human colon. We have also used acetyl-beta-methyl choline, a choline ester, which, because of its slower hydrolysis by cholinesterase, is effective after subcutaneous injection. This drug also differs qualitatively from acetylcholine in that it possesses muscarinic action only, lacking the nicotinic action of acetylcholine (6).

MATERIALS AND METHODS

Eighteen paid volunteer college students and medical students were the subjects for the experiments in which acetylcholine was administered. All were male and they ranged in age from 18 to 32 years. Methacholine was given to medical students, patients with diseases unrelated to the bowel, and patients with the irritable colon syndrome. These subjects ranged in age from 16 to 55 years; 16 were male, eight were female. The nature of the procedure and the subjective effects of the drug to be used were carefully and reassuringly explained to each individual prior to the experiment.

Sigmoid motility was recorded by a simple balloon-kymograph technique previously described in detail (7). As preparation, each subject fasted six to 16 hours and the colon was emptied by one or two enemata of physiological saline two hours before the experiment. During a 30 to 60 minute control period, a sigmoid tracing was recorded and the blood pressure and pulse rate were measured by the usual clinical methods approximately every five minutes.

Methacholine [3] (acetyl-beta-methyl choline chloride) was administered subcutaneously, 2.5 to 10 mgm. It was usually repeated in a larger or smaller dose within this range at least once in each subject. After the injection, blood pressure and pulse rate were recorded each minute until all signs and symptoms of drug effect had disappeared. The subject was carefully observed for other systemic effects and their presence or absence was recorded. Each subject received one or more injections of physiological saline as control, after which similar observations were made.

Acetylcholine was administered by continuous infusion at accurately controlled rates. A sterile solution of acetylcholine chloride in saline containing 100 mgm. of acetylcholine per cc. was prepared and stored in the refrigerator. From this stock solution the final dilution (5 or 10 mgm. per cc.) was prepared just prior to its use. The infusion apparatus consisted of two 250 cc. burettes, one filled with physiological saline, the other with the acetylcholine solution. These led to a three way stop-cock bearing the intravenous needle. The rate of flow was controlled by a tunnel clamp and a glass infusion meter [4] placed in each system. A Murphy drip was also included in each system as an additional guide to the rate of flow. The actual amount of solution in-

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[3] The methacholine chloride and acetylcholine chloride were generously supplied by Dr. M. Carlozzi of Merck & Co., Inc., Rahway, N. J.

[4] Flowrator (Graubard-Peterson) manufactured by Fischer & Porter Co., 350 Fifth Avenue, New York, N. Y.

fused each minute was read from the burette at intervals of one or two minutes.

Shortly after starting the sigmoid motility tracing, the infusion was initiated with saline and continued for at least 30 minutes before the acetylcholine was administered. During this control period, blood pressure and pulse rate were recorded every five minutes, and during the acetylcholine infusion at one minute intervals, while detailed notes were made of the rapidly changing systemic effects upon the subjects. The infusion was begun slowly and the rate of flow increased stepwise. Increases in rate were halted when the subject experienced a moderate degree of respiratory distress and tachypnea. The intensity of these respiratory symptoms was approximately the same in all subjects. The infusion rate was then maintained constant, usually for five to eight minutes. The entire procedure lasted 10–15 minutes. If the rate of flow was increased too rapidly, unpleasant or potentially dangerous side effects occasionally developed, at which time the infusion was immediately terminated.

Subcutaneous methacholine and intravenous acetylcholine were also given to a number of subjects while fluoroscopic observations of the barium filled colon were being made. "Spot" films were taken of the cecum and sigmoid before drug administration and several times during the period of drug effect.

RESULTS

Effect of acetylcholine on the colon. Eighteen subjects received 21 acetylcholine infusions. In 15 of these experiments there was complete or almost complete abolition of sigmoid contractions (Figure 1); in two experiments, there was a definite increase in contractile activity, and no detectable change occurred in four.

Acetylcholine infusions were administered to three subjects during fluoroscopic observations of the colon after it had been cautiously filled, but not distended, with barium sulphate suspension by enema. In two of these experiments, the right half of the colon was seen to contract vigorously, with simultaneous shortening, widening and disappearance of segmental contractions in the sigmoid and distal descending colon (Figure 2).

The effect of acetylcholine on the colon became apparent only when the rate of infusion was such that the other systemic effects were fully established. These effects were no less pronounced in subjects whose colon motility pattern was not altered.

Other effects of acetylcholine. As the acetylcholine infusion was initiated, the patient usually experienced a burning sensation at the site of the needle, which extended along the course of the brachial veins into the shoulder. The superficial veins often became dilated and purplish red. This pain became very severe at times, particularly when a drug concentration of 10 mgm. per cc. was used, but it always disappeared after two to three minutes, despite an increasing rate of infusion.

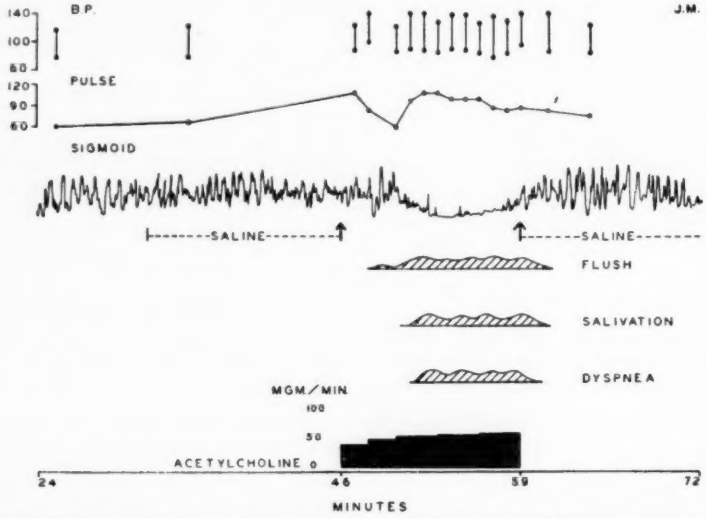

FIG. 1. CHANGES IN SIGMOID MOTILITY DURING AN INFUSION OF ACETYLCHOLINE IN A NORMAL SUBJECT

As the stepwise increases in rate continued, there developed a feeling of warmth over the face, and to a lesser extent elsewhere, associated with a flush limited to the face, the neck, and occasionally the chest. These were often, but not always, followed by variable amounts of visible sweating, and a slight increase in salivation. In some subjects there was slight lacrimation. As the rate was further increased respiratory difficulty, tachypnea, and often a desire to cough became manifest. It was at this rate of flow that the infusion was maintained. Despite the constant rate, there was considerable fluctuation of all signs and symptoms; the blood pressure and pulse rate varied unpredictably. There was an initial irregular rise in systolic and diastolic blood pressure in almost all subjects, followed by a fall below the control level in 13; often there was a fall in diastolic pressure only, with an increase in pulse pressure. In general the pulse rate varied directly with the diastolic blood pressure. In a few subjects there was no significant change in the blood pressure or pulse. If the rate of infusion was increased too rapidly, or if the level of tolerance was exceeded, hypotension, bradycardia, nausea, and occasionally uncontrollable coughing developed. A number of subjects experienced central nervous system effects, manifested by feelings described as "far away from everything" or "I am going to faint." These were not related to blood pressure or heart rate changes. After termination of the infusion all symptoms subsided abruptly and disappeared completely within two or three minutes.

The amount of acetylcholine necessary to achieve the desired level of drug effect varied from 40 to 120 mgm. per minute with an average of 72. The total amount of acetylcholine given during an infusion varied from 505 to 1,270 mgm., averaging 739 mgm. The effective amount was independent of the weight of the individual.

Comment. This wide variation in individual tolerance to acetylcholine is in accord with the experience of Ellis and Weiss (8, 9). These authors infused acetylcholine intravenously into normal men and recorded systemic effects similar to those observed in these experiments. Carmichael and Fraser (10) also administered acetylcholine intravenously to human beings, but because of a different technique of administration their results are not comparable.

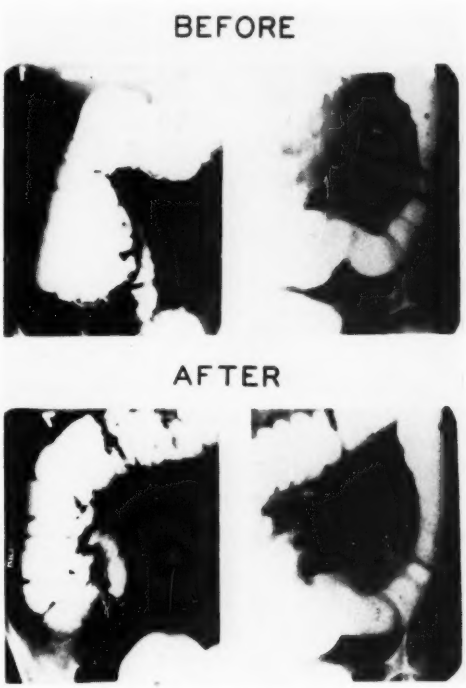

FIG. 2. ROENTGENOLOGIC APPEARANCE OF THE RIGHT AND LEFT PORTIONS OF THE COLON BEFORE INFUSION OF ACETYLCHOLINE AND AT THE HEIGHT OF THE EFFECT OF THIS AGENT

Effect of methacholine on the colon. Methacholine was administered 49 times to 24 subjects. In 20 of these experiments, there followed a marked diminution or complete absence of wave-like motility of the sigmoid (Figure 3). In six experiments the results were inconclusive, and in 23 experiments there was no significant change after methacholine. In no instance did methacholine produce augmentation of sigmoid motility.

In five of the subjects in whom methacholine produced abolition of contractions of the sigmoid, the injection of methacholine was followed by the intravenous administration of 0.6–1.0 mgm. of atropine sulphate. This caused only a brief interruption of sigmoid contractions, followed by a prompt return to the previous pattern. Thereupon the same or a larger amount of methacholine was again administered, and its effect upon sigmoid motility was blocked in each instance (Figure 4).

One subject with a transverse colostomy re-

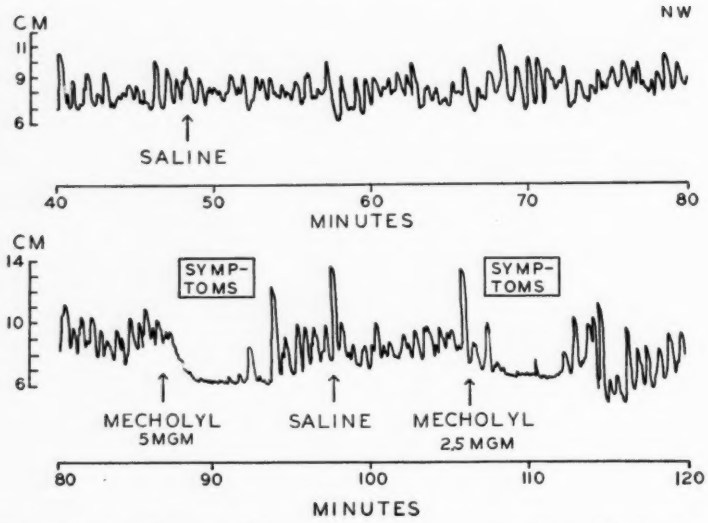

FIG. 3. TYPICAL EFFECT OF SUBCUTANEOUS METHACHOLINE (MECHOLYL®) UPON THE MOTILITY OF THE SIGMOID COLON

Control injections of saline produce no effect.

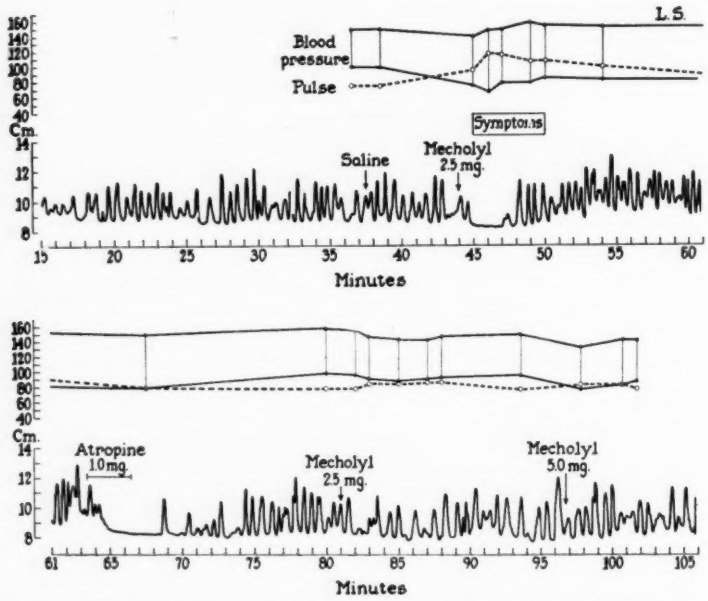

FIG. 4. CONTINUOUS RECORD OF HUMAN SIGMOID MOTILITY, DEMONSTRATING THAT AFTER INTRAVENOUS ATROPINE, METHACHOLINE FAILS TO AFFECT THE SIGMOID MOTILITY, THE PULSE, OR THE BLOOD PRESSURE

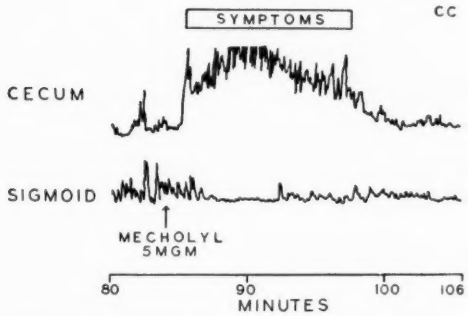

FIG. 5. BALLOON-KYMOGRAPHIC RECORDINGS OF MOTILITY OF THE CECUM AND SIGMOID COLON, SHOWING COORDINATE ACTION OF METHACHOLINE ON THESE REGIONS OF THE BOWEL

ceived methacholine while motility tracings were being recorded simultaneously from a balloon in the sigmoid and a balloon in the cecum. There was a pronounced increase in activity in the cecum, resulting in the expulsion of feces from the colostomy, coincident with the abolition of sigmoid contractions (Figure 5).

Methacholine was administered subcutaneously to six subjects while the barium filled colon was observed fluoroscopically. In four of these, the right half of the colon contracted forcefully, while the distal colon shortened and widened. This response was identical with that produced by acetylcholine.

Other systemic effects of methacholine. The effects of methacholine on other structures were similar to those produced by acetylcholine. The flush was usually more intense, lacrimation more prominent, and respiratory embarrassment less marked. The blood pressure and pulse rate almost invariably rose to some extent. These systemic effects of subcutaneously administered methacholine have been described repeatedly (3, 5, 6, 11), with a wide variation in individual sensitivity to the drug. This individual difference in intensity of effect, as well as the absence of effect on the colon in 23 experiments, may have been partly due to an irregular rate of absorption of the subcutaneously injected drug.

The alteration in sigmoid motility was not related to the intensity of these phenomena, but it was during their presence that the flattening of the tracing occurred. The subjects were never aware of abdominal cramps or intestinal activity.

DISCUSSION

In these experiments the usual effect of either acetylcholine or methacholine on the human colon was (*1*) abolition of phasic activity of the circular muscle of the sigmoid, (*2*) shortening and widening of the same segment, and (*3*) increased propulsive activity of the more proximal portions of the colon. These findings are compatible with those of Abbott (11) who found an increase in the overall pressure within the fluid-filled human colon of three out of six subjects following the injection of 5 to 15 mgm. of methacholine. Yet, they would seem to contradict extensive studies on the actions of these drugs in animals, in which they have augmented intestinal motility at all levels examined. This discrepancy can probably be explained by the difference in the anatomical and functional organization of the colon in man and in the usual herbivorous or carnivorous laboratory animals (12).

The stimulation of the intestine produced by acetylcholine in animals is considered a muscarinic effect. When intestinal motility in the atropinized animal is abolished by acetylcholine, the effect is regarded as due to its synaptic (nicotinic) action, with increased sympathetic stimulation of the gut. In our observations on the human sigmoid, however, both acetylcholine and methacholine abolished phasic activity. In the case of acetylcholine, this effect could be due either to its synaptic or to its muscarinic action, but since methacholine possesses only a muscarinic action, the response to this drug probably represents direct cholinergic stimulation of the smooth muscle. This conclusion is strengthened by the fact that the response was blocked by atropine. With both drugs, the reduction of sigmoid motility coincided with forceful contractions in the right colon and with extraintestinal signs of muscarinic activity, such as salivation, flushing of the skin, and usually a fall in blood pressure.

These coordinate actions of acetylcholine and methacholine on the proximal and distal colon seem appropriate for the propulsion of fecal masses and for the reception of these masses in the lower portions of the bowel. Hurst (13), observing the propulsion of feces in man by fluoroscopy, noted simultaneous abolition of circular muscle activity of the distal colon, shortening of the same segment, and massive contractions beginning

either in the right colon or in the region of the splenic flexure. Galapeaux, Templeton, and Borkon (14), in producing diarrhea in dogs by the instillation of bile into the colon, observed a reduction in phasic activity of the distal colon. The same phenomenon has been observed in kymographic studies on the human sigmoid in patients with functional diarrhea (15). Moreover, in a recent study of patients with ulcerative colitis (16), those patients with severe diarrhea were found to have an absence or marked reduction of sigmoid contractions, while those without diarrhea had normal sigmoid motility. The similarity of the sigmoid motility patterns in diarrheal diseases with those induced by cholinergic stimulation suggests that sustained parasympathetic activity may be involved in the pathogenesis of these conditions.

SUMMARY

The effect of acetylcholine and methacholine upon the colon of normal individuals has been studied, using a balloon-kymograph technique to record the motility of the sigmoid, and (in one experiment) of the cecum. Acetylcholine was administered intravenously 21 times to 18 subjects, and methacholine was injected subcutaneously 49 times in 24 subjects. In 20 experiments with methacholine, and in 15 experiments with acetylcholine, these drugs abolished the phasic contractions of the sigmoid. Acetylcholine augmented sigmoid contractions in two experiments, and methacholine in none.

In nine experiments, the entire colon was observed by fluoroscopy and spot roentgenograms. In these, at the time of disappearance of sigmoid contractions, the sigmoid appeared to shorten and the right colon contracted vigorously. This coordinate pattern of motor activity, produced by cholinergic drugs, is similar to that which occurs spontaneously during mass peristalsis.

The sigmoid motility response to acetylcholine and methacholine is identical with that observed in association with diarrhea in man and animals. This may help to define the role of the parasympathetic nervous system in the mechanism of functional diarrhea and of ulcerative colitis.

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The authors are indebted to Miss Natalie Stolk for invaluable technical assistance.

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PLASMA FIBRINOGEN AND THE SEDIMENTATION RATE IN RHEUMATOID ARTHRITIS, AND THEIR RESPONSE TO THE ADMINISTRATION OF CORTISONE AND ADRENOCORTICOTROPIC HORMONE (ACTH) [1]

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The purpose of this communication is to report on our investigations into the relationship between the erythrocyte sedimentation rate and the level of the plasma fibrinogen in patients with rheumatoid arthritis, and to describe the effect of the administration of cortisone and ACTH upon this relationship. It will also show that the sedimentation rate often fails to reflect the high rebound levels reached by the blood fibrinogen during the relapse period which follows the abrupt termination of treatment with these hormones.

HISTORICAL

In 1918 and in 1921 Fahraeus (1) drew attention to a new form of clinical examination of the blood, the determination of the sedimentation velocity of the red blood corpuscles. Since that time this determination has been used extensively in the investigation of patients, and has been shown to be of particular value in assessing the intensity or the continued activity of a number of disease processes. Much investigation has been carried out in an effort to determine the factors responsible for the changes in the sedimentation rate. Fahraeus (2) and many others have pointed out that the erythrocyte settling velocity may be affected by a number of factors. These include the size, shape, and numbers of the red blood cells, the viscosity of the plasma, the plasma content of cholesterol, lecithin, nucleoprotein, and the presence in the blood of abnormal globulins or other substances having a large asymmetric molecular configuration. In his review of 1929, Fahraeus (2) expressed the opinion that although the sedimentation rate can be influenced by many factors, it is in the main an expression of the size of the particles formed by the aggregated rouleaux of the red blood cells, when clotting is prevented. He also expressed the belief that while the size of these particles is determined by certain changes in the surface layer of the erythrocytes, these changes are dependent upon the properties of the blood plasma rather than upon any intrinsic alteration in the cells themselves. He stated that the principal factor responsible for this clumping effect is the concentration of the plasma fibrinogen, and that in most cases a marked parallelism exists between the amount of fibrinogen present and the sinking velocity of the erythrocytes. A similar but less marked influence is exerted by the plasma globulin. These observations and opinions of Fahraeus have been supported by many investigators and challenged by others (3).

In 1938, Ham and Curtis (4) reviewed the significance of the sedimentation rate as a laboratory aid in clinical medicine. They concluded that the rate of settling of the red blood cells, as determined by the method of Rourke and Ernstene (5), showed a rough linear correlation with the concentration of plasma fibrinogen, providing that the serum globulin, the mean corpuscular volume, and the corrected defibrinated sedimentation rate were all within normal limits. The degree of correlation was only slightly less when the Westergren (6) method was used.

Gray and Mitchell (7), by the addition of various electrophoretically homogeneous fractions of plasma to blood, have demonstrated that the accelerating effect of fibrinogen upon the sedimentation rate is roughly six times that of serum globulin.

[1] This study was assisted by a grant-in-aid from the Canadian Arthritis and Rheumatism Society.

[2] Canadian Arthritis and Rheumatism Society Fellow in Medicine.

Gordon and Wardley (8) made similar observations but noted that the relationship between the plasma fibrinogen and the sedimentation rate was not always a simple one. They suspected the existence of a complex inhibiting influence of one protein upon another.

In 1946, Malmros and Blix (9) published observations made over a period of eight years in patients showing high sedimentation rates and suffering from a variety of disorders. The globulins were estimated by the electrophoretic technique. They recognized the difficulty of correlating the sedimentation rate with the individual constituents of the plasma, and stated that the correlation would vary according to the clinical material used. For example, they reported cases of hyperglobulinemia of unknown origin, where the sedimentation rate was very high and the fibrinogen content of the plasma was normal. Similar observations were made in myelomatosis, nephrosis, and in liver disease.

It is well known that there is an increase in the plasma fibrinogen in many disease states, notably those of infection, inflammation and tissue injury or necrosis. Experimentally this response is inhibited or abolished when the liver is severely damaged, and it has been assumed that the liver is the source of the increased fibrinogen (10, 11).

There is general agreement that in patients suffering from rheumatoid arthritis, the settling velocity of the red blood cells is an indication of the activity of the disease process, perhaps more so than in any other illness. Earlier studies by the senior author (12) on the plasma protein fractions in this disease have shown that, in the active stages, the elevated sedimentation rate is almost always accompanied by an increase in the level of the plasma fibrinogen, and in the majority of instances the serum globulins are not seriously altered.

Hench, Kendall, Slocumb, and Polley (13), in announcing the dramatic effect of cortisone and ACTH on the course of rheumatoid arthritis, stated that during the remission there was an abrupt fall in the sedimentation rate and that it rose again as the arthritis relapsed when the administration of the hormones was discontinued.

A year later, Vaughan, Bayles and Favour (14) reported that during the remissions induced by these hormones in rheumatoid arthritis, there occurred a rapid fall in the plasma fibrinogen and that this fall closely paralleled the fall in the sedimentation rate. They also noted a fall in the concentration of the gamma globulin but this was found to occur more slowly and usually lagged five to seven days behind the fibrinogen changes. In another paper (15) these investigators concluded that the decrease in the plasma fibrinogen and gamma globulin was responsible for the observed changes in the sedimentation rate. It seemed to them that the difference in the rates of response of these proteins to hormone therapy was probably due to differences in the rates of synthesis.

Ogryzlo and Graham (16) reported that in cases of Reiter's syndrome and rheumatoid arthritis treated with ACTH or cortisone, the level of plasma fibrinogen closely paralleled the clinical activity of the disease process and bore a close relationship to the erythrocyte sedimentation rate.

Fischel (17) has also stated that the depression of fibrinogen and gamma globulin, during hormone therapy, is not necessarily a primary effect of the hormone but appears more likely to be a reflection of the effect of the hormones on the inflammatory disease process present in the patients studied.

MATERIALS AND METHODS

The observations reported in this communication are based on the study of the level of the plasma fibrinogen and erythrocyte sedimentation rate in 160 patients with rheumatoid arthritis selected at random from the arthritis service of Sunnybrook (D.V.A.) Hospital, Toronto, since 1948. Three of these patients have been studied carefully in this way over a three year period. Altogether the sedimentation rate has been compared with the plasma fibrinogen in over 1,000 instances.

With the advent of ACTH and cortisone, remissions may now be induced in such patients almost at will and over short periods of time. Such remissions have afforded an excellent opportunity for the further study of the relationship between the sedimentation rate and plasma fibrinogen. In the past two years this relationship has been followed at frequent intervals in 18 patients, both during the induction of the remissions and the subsequent relapses. A total of 27 such courses of therapy was studied.

Plasma fibrinogen has been determined by the method of Cullen and Van Slyke (18). The serum protein fractions have been separated by the sodium sulphite procedure of Campbell and Hanna (19) and quantitatively measured by the biuret technique of Gornall, Bardawill,

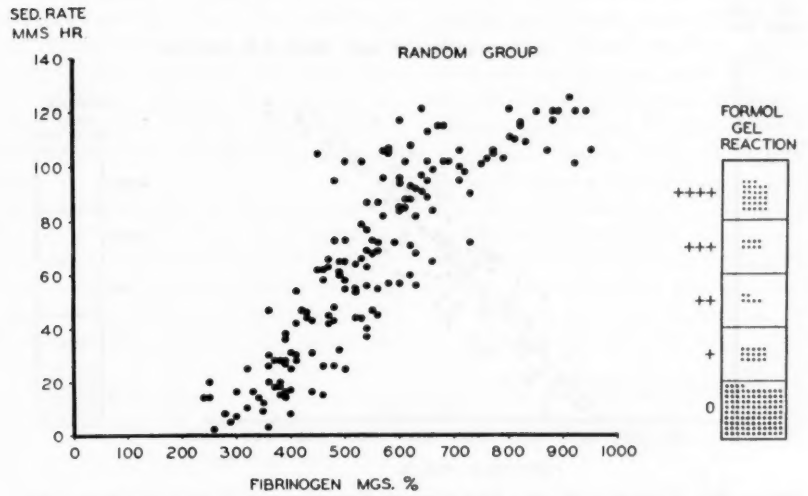

FIG. 1. RELATIONSHIP OF PLASMA FIBRINOGEN TO SEDIMENTATION RATE IN RHEUMATOID ARTHRITIS

Coefficient of linear correlation, r = 0.869. The qualitative serum formol gel reactions are also shown. (Formol gel reactions are recorded as follows: 0 = no obvious change in viscosity after 24 hours; + = mild to moderate increase in viscosity after 24 hours; ++ = marked increase in viscosity but no definite gel after 24 hours; +++ = definite gel in 24 hours but gel is soft and not rigid; ++++ = serum is a solid stiff gel in 24 hours or less.)

and David (20). The formol gel reaction (21), thymol turbidity, cephalin cholesterol flocculation and hemoglobin concentration were also determined. Gamma globulin was determined in a number of cases by Kunkel's flocculation method (22). The results of many of these latter observations will be considered in a subsequent report. The sedimentation rate has been determined by a modified Westergren method, 0.05 cubic centimeter of a 30 per cent solution of potassium oxalate being added to each 5 cubic centimeters of blood as an anticoagulant. Much care has been exercised to establish uniformity of technique.

RESULTS

Normal levels of plasma fibrinogen by the method employed have varied from 240 to 360 milligrams per cent. The normal range given by Ham and Curtis (23) was 190–330 milligrams per cent. In active rheumatoid arthritis the fibrinogen content of the blood is almost always raised, and levels as high as 700 or 800 milligrams per cent are not infrequently observed. The highest level reached in this present study was 1,130 milligrams, during a period of relapse following a course of administration of cortisone.

The relationship of the plasma fibrinogen to the sedimentation rate in rheumatoid arthritis is shown in Figure 1, where the fibrinogen content of the blood has been plotted against the sedimentation rate. In this scattergram there is a rough linear correlation between the fibrinogen concentration and the sedimentation rate. Fibrinogen levels from 250 to 350 milligrams per cent are associated with a sedimentation rate below 20 millimeters in one hour. Concentrations of fibrinogen above this level are associated with progressively higher rates, reaching a maximum of 100 millimeters or over as the blood fibrinogen rises to 600 or 700 milligrams per cent. As is apparent, there is a considerable spread in the position of individual dots representing this relationship for any one level of fibrinogen. Other factors besides the fibrinogen content must influence the settling velocity of the cells and consequently the exact position of the dots. The spread appears much more marked for the high rates of sedimentation.

When patients with levels of total serum globu-

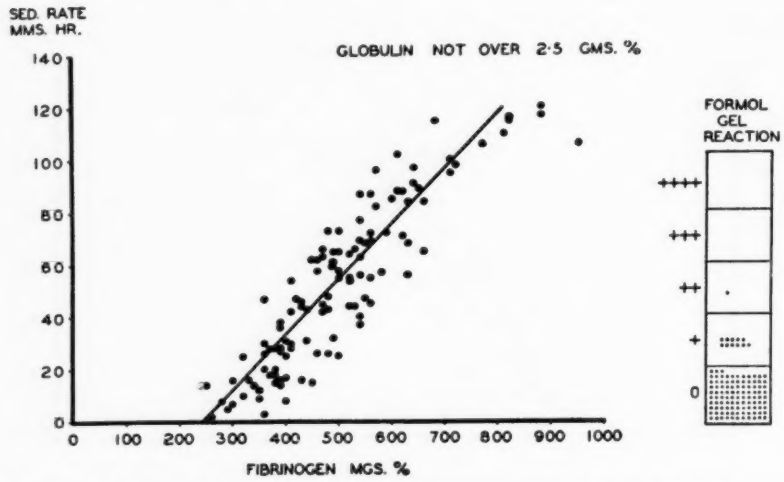

FIG. 2. RELATIONSHIP OF PLASMA FIBRINOGEN TO SEDIMENTATION RATE IN PATIENTS WITH SERUM GLOBULIN VALUES NOT EXCEEDING 2.5 GRAMS PER CENT

Coefficient of linear correlation, r = 0.897.

lin over 2.5 grams per cent (as determined by precipitation with 19 volumes of 21 per cent sodium sulphite) are excluded from the group, the resulting scattergram exhibits a better correlation (Figure 2). Much of the spread at the higher rates of sedimentation is eliminated. Considerable spread, however, persists and analysis of our data has shown that it is not diminished by allowing for variations in the hemoglobin content of the blood. Four or five patients with hemoglobin

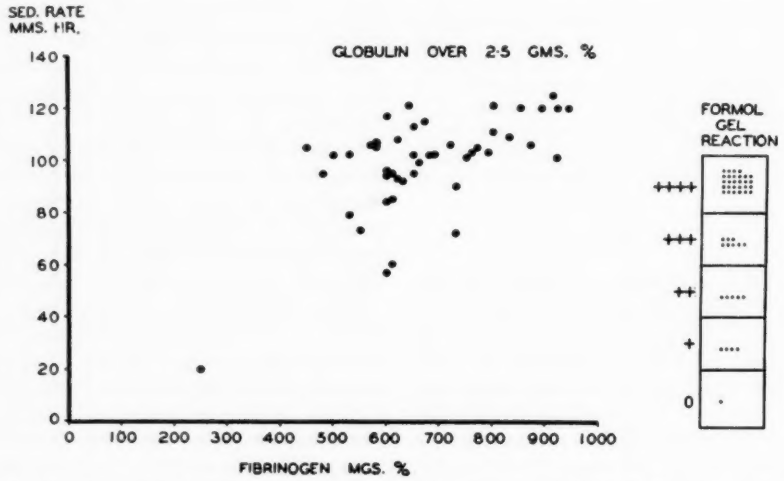

FIG. 3. RELATIONSHIP OF PLASMA FIBRINOGEN TO SEDIMENTATION RATE IN PATIENTS WITH SERUM GLOBULIN VALUES EXCEEDING 2.5 GRAMS PER CENT

Coefficient of linear correlation, r = 0.599.

levels below 9.9 grams per 100 cubic centimeters were excluded from this series. The coefficient of linear correlation for this group of patients is high ($r = 0.897$).[3] The regression line runs from about 250 milligrams per cent of fibrinogen at a sedimentation rate of 0 millimeter, and reaches 700 milligrams per cent at a sedimentation rate of 100 millimeters.

In Figure 3, a scattergram is shown for patients in whom the serum globulin is in excess of 2.5 grams per cent. Here the correlation is not nearly as good, but it may be pointed out that in this group (with one exception, globulin 2.6 grams per cent) the sedimentation rate was high, and in the majority of instances was over 80 millimeters in one hour. While it is evident in these that the globulin is playing some part in the accelerated rate of sedimentation, in most of them the fibrinogen is also raised and the effect of the globulin is to a large degree masked by the elevated fibrinogen.

In Figures 2 and 3 the results of the qualitative serum formol gel reaction are shown. When the globulin level is below 2.5 grams per cent, this reaction has usually been negative or occasionally weakly positive. When the globulin rises above 2.5 grams per cent, this reaction begins to become positive and at levels above 3.0 grams per cent is almost always strongly positive. This is in agreement with the statement made that the formol gel reaction may be employed to obtain a rough estimation of the degree of hyperglobulinemia (24).

A typical response of the blood fibrinogen and the sedimentation rate to the administration of ACTH is shown in Figure 4. This occurred in a case of severe rheumatoid arthritis of ten years duration. The patient was a 35 year old army veteran who had had no previous remission during his three year stay in hospital, despite prolonged treatment with bed rest, various physical measures and the administration of gold salts. ACTH, 100 milligrams daily, was given in divided doses over a period of 24 days. During this time the patient underwent an almost complete clinical remission. However, as soon as the treatment was stopped he began to relapse and within seven days his condition had returned to its original severity. During the remission, as is shown in Figure 4, the plasma fibrinogen fell from 660 milligrams per cent to 370 milligrams per cent in the first seven days, and to 240 milligrams per cent after 14 days. When the ACTH was discontinued, the fibrinogen level rose rapidly and abruptly to pretreatment levels within eight days

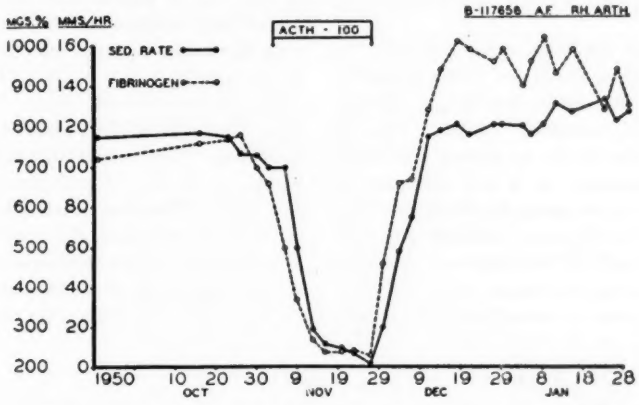

FIG. 4. THE EFFECT OF A 24 DAY COURSE OF ACTH ON THE SEDIMENTATION RATE AND PLASMA FIBRINOGEN IN A PATIENT WITH RHEUMATOID ARTHRITIS

Note the high rebound of the plasma fibrinogen.

[3] The authors are indebted to A. J. Phillips, Ph.D., statistician, National Cancer Institute of Canada, for the statistical analysis of this report.

time, and continued to rise until it reached 1,010 milligrams per cent, 14 days later. From this level it gradually dropped to the pretreatment figure.

In the figure, the sedimentation rates are plotted in such a way that a value of 100 millimeters per hour will correspond to a fibrinogen level of 700 milligrams per cent, and a rate of 0 millimeters to a fibrinogen of 200 milligrams. The correlation in this case when this is done is very close indeed, both during the remission and during the relapse periods. When the sedimentation rate reached 100 millimeters, however, it failed to follow the rising fibrinogen level, and for the next six weeks did not reveal the high rebound figures reached by the fibrinogen.

During both the remission and the relapse, the change in the plasma fibrinogen preceded that of the sedimentation rate by 24 to 48 hours, although the curves remain essentially parallel. This lag phenomenon results in the temporary dislocation of the interrelationship. It is well illustrated in Figure 4, where at one point the fibrinogen level has fallen to 500 milligrams, although the sedimentation rate is still 100 millimeters. A similar lag is observed during the relapse, when a fibrinogen of 460 milligrams is associated with a sedimentation rate of only 20 millimeters. Obviously single estimations made during times of rapid change will fail to reveal the close interrelationship.

The various relationships during the induction of two remissions and two relapses in the same patient are plotted in scattergram fashion in Figure 5. The round and square closed dots represent two successive remissions; the round and square open dots, two successive relapses. The line joining the dots, representing the successive relationships between the fibrinogen and sedimentation rates at various stages of the remission and relapse, is seen to follow a definite pattern. With the onset of a remission there occurs initially a decrease in the level of plasma fibrinogen without a corresponding decrease in the sedimentation rate, with the result that the curve moves to the left of the regression line. After 24 to 48 hours, the sedimentation rate also begins to fall and thereafter they both fall in such a way that the curve indicating their interrelationship passes downwards, to the left of and parallel to the regression line. When low levels are reached and the remission is complete, the dots return to the regression line. When treatment is stopped and the relapse begins, the situation is seen to be reversed. At the beginning of the relapse fibrinogen is in-

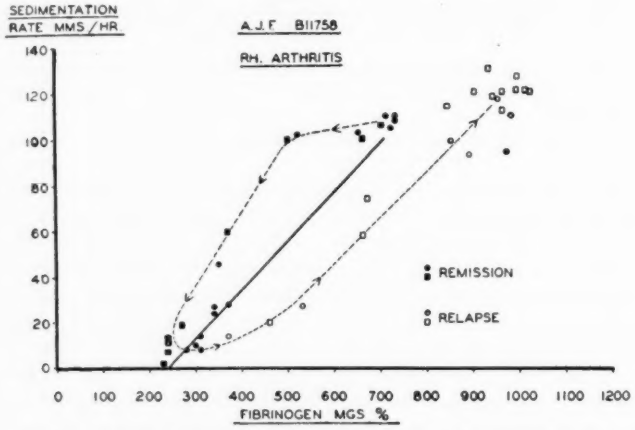

FIG. 5. THE PROGRESSIVE INTERRELATIONSHIPS OF SEDIMENTATION RATE AND PLASMA FIBRINOGEN DURING TWO SUCCESSIVE REMISSIONS AND RELAPSES INDUCED BY ACTH AND CORTISONE IN A PATIENT WITH RHEUMATOID ARTHRITIS

The course of the curve has the form of the letter "C."

creased without there being any change in the sedimentation rate. As a result the curve moves to the right. It then rises roughly parallel to the regression line. Finally a group of dots at the end of the curve show the high levels of fibrinogen that were observed during the rebound period.

The curve thus obtained has the appearance of the letter "U" or the letter "C." The shape of the curve is similar in all cases when such a remission is induced. The curve may be largely or entirely to the left or to the right of the regression line depending upon the initial relationship of the fibrinogen and the sedimentation rate. The downstroke and upstroke of the curve run parallel or nearly so to the regression line.

Figures 4 and 5 both demonstrate that when the blood fibrinogen rises above 700 milligrams per cent, a further increase is not indicated by the sedimentation rate. This of course is not unexpected, since a rate of 100 millimeters in one hour, in the absence of anemia, has already nearly reached its maximum speed. The pattern of the change in most patients studied has been uniformly similar, with few minor variations (Figure 6).

In contrast to the above observations, the changes occurring in the levels of the serum euglobulin (as measured by precipitation with 19 volumes of 15 per cent sodium sulphite), and the gamma globulin (as estimated by Kunkel's flocculation method), are of a different order and roughly parallel one another. Both fail to show any close correlation to the rate of erythrocyte sedimentation. During the onset of a remission the euglobulin and gamma globulin concentrations both fall, but the decrease is more gradual than that which occurs in the fibrinogen level (Figures 7 and 8). Furthermore both of these constituents may continue to fall for an appreciable period after hormone therapy has been discontinued, even though the fibrinogen and the sedimentation rate have started to rise and clinical relapse has begun. Eventually, however, the euglobulin and gamma

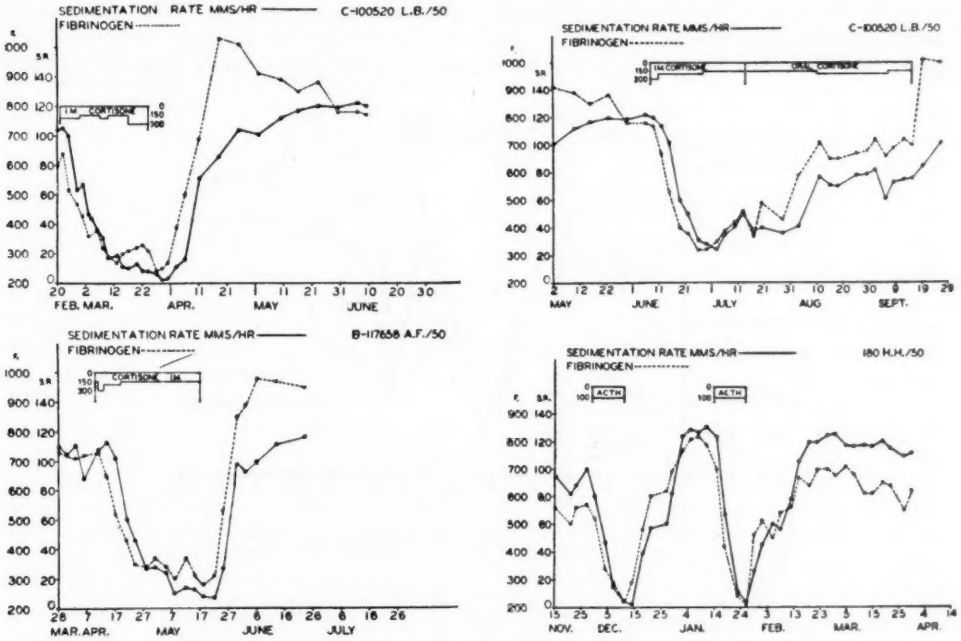

FIG. 6. FOUR ILLUSTRATIVE CASES SHOWING THE EFFECT OF CORTISONE AND ACTH ON THE PLASMA FIBRINOGEN AND SEDIMENTATION RATE IN RHEUMATOID ARTHRITIS

Note that the fall in fibrinogen precedes that of the sedimentation rate in all instances.

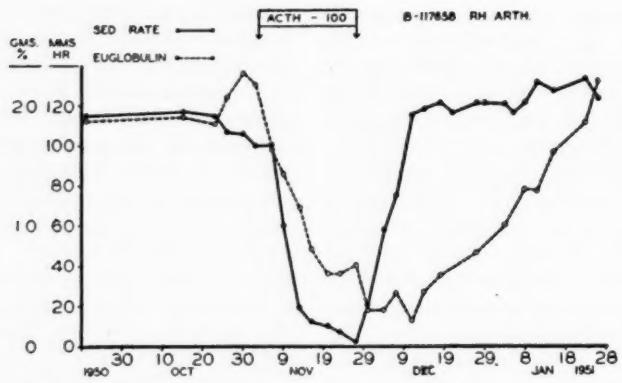

FIG. 7. THE EFFECT OF A 24 DAY COURSE OF ACTH ON THE SEDIMENTATION RATE AND SERUM EUGLOBULIN IN A PATIENT WITH RHEUMATOID ARTHRITIS

Note that the changes in the euglobulin lag behind those in the sedimentation rate. The two curves show no parallelism. Compare with Figure 4.

globulin return to their pretreatment levels, or may temporarily exceed these. Not infrequently the euglobulin may drop to levels considerably below normal, a feature rarely observed in regard to the plasma fibrinogen. In Figure 7 the euglobulin fell from an abnormally high level of 2.27 to 0.21 gram per cent, as compared with a normal range of 0.5 to 1.0 gram per cent. After withdrawal of hormone therapy the euglobulin rose slowly and reached pretreatment levels about two months

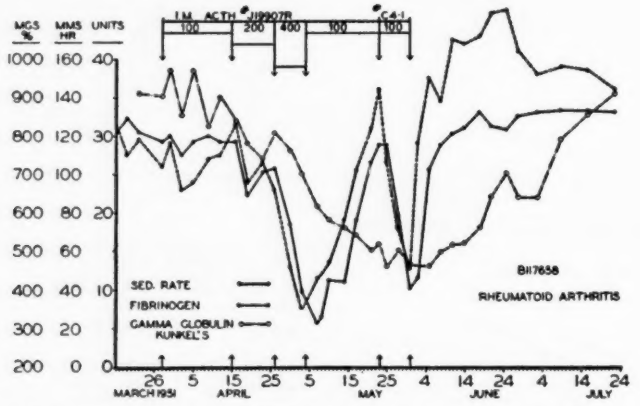

FIG. 8. THE EFFECT OF ACTH ON THE SEDIMENTATION RATE, PLASMA FIBRINOGEN AND THE GAMMA GLOBULIN IN A PATIENT WITH RHEUMATOID ARTHRITIS

A typical remission was induced with 400 milligrams daily of a preparation of low potency, but clinical relapse occurred when this was reduced to 100 milligrams daily. Note that the fibrinogen and sedimentation rate parallel the clinical course, while the gamma globulin fails to indicate the relapse in the disease. A further remission took place with the administration of an active preparation in the same dosage.

later. As shown in Figure 8, a clinical relapse occurred while the patient was receiving 100 milligrams daily of an ACTH of low potency. The relapse was reflected closely by the sedimentation rate and the plasma fibrinogen, but it was not reflected by the gamma globulin, presumably because this globulin is not capable of indicating rapid changes in the clinical state.

DISCUSSION

Our observations made prior to the introduction of cortisone and ACTH have been in agreement with those of Fahraeus, and of Ham and Curtis; *viz.*, that the sedimentation rate is a rough index of the blood fibrinogen level in patients with rheumatoid arthritis when there is no abnormality in the globulin content and in the absence of severe anemia. The raised fibrinogen level in turn appears to be a reaction to the inflammatory process or tissue injury. The clinical material studied contains a number of early and comparatively mild cases, the large majority of which showed little or no globulin elevation. In these the sedimentation rate seemed to be an index of the intensity of the disease reaction rather than one of the severity or gravity of the disease itself. In this way it is comparable to the significance of fever or leucocytosis. Even when the globulin level is raised (or when the serum formol gel reaction is strongly positive), the high sedimentation rates of rheumatoid arthritis will, as a general rule, be associated with an elevated plasma fibrinogen.

A scattergram showing the various fibrinogen concentrations plotted against the sedimentation rate has provided an average regression line indicating the interrelationship and also shows the degree of deviation which may occur.

With the fall in the plasma fibrinogen and sedimentation rate which accompanies a partial or complete remission induced by ACTH or cortisone in patients with rheumatoid arthritis, the relationship of the sedimentation rate and the plasma fibrinogen is to a large extent maintained, except that there is a lag in the sedimentation rate fall of 24 to 48 hours. The lag is also observed during the relapse period which sets in on withdrawal of the hormone. This results in a temporary but quite material dislocation in the relationship during the time of rapidly changing levels. This dislocation of the interrelationship is not inconsistent with present theories of the mechanism underlying the sedimentation rate. The increased settling velocity of the red cells appears to be directly related to the size of the particles of aggregated rouleaux and the formation of these large particles is thought to be dependent upon the increased adhesiveness or stickiness of the red cells brought about by the large fibrinogen or globulin molecules. This theory has been discussed in detail by Ponder (25). In the light of this conception it would seem likely that the red cells are not immediately influenced by changing blood fibrinogen, and that some time interval must elapse before the cells assume the adhesiveness which should correspond to a new concentration of the plasma protein. The course of the sedimentation rate during rapid changes of blood fibrinogen might then be said to offer material support to this concept of their interrelationship.

It has also been shown that, during the relapse which sets in when hormone administration is stopped, the blood fibrinogen may rise to as high as 50 per cent above pretreatment levels. These rebound figures appear to indicate an actual increase in the intensity of the arthritic process. In one patient an intractable iridocyclitis developed at this time. Other patients also have appeared worse during the rebound period than before initiating hormone therapy. The occurrence of such reactions constitutes a possible menace and might be a warning against the abrupt cessation of treatment in severe cases.

The significance of the behavior of blood fibrinogen following hormone therapy has been a subject of some controversy. One might expect that the level of this protein would continue to be an index of tissue inflammation or injury and would reflect the hormonal suppression of the intensity of the disease. Recently Vaughan, Bayles, and Favour (15) have questioned this view and have stated that it is also reasonable to assume that the changes in protein levels are the result of direct action of the cortical steroids upon their production. Such an effect would be consistent with the anti-anabolic properties of these hormones. It does seem that at times this anti-anabolic effect may be evident and the fibrinogen level may fall beyond what might be expected in the light of the

degree of clinical improvement. However, this effect appears to be a minor one when the levels of fibrinogen are changing rapidly. As a rule the level of the fibrinogen follows fairly well the course of the remission and the relapse. In exceptional cases where there is a poor clinical response, there is only a partial fall in the blood fibrinogen. The high figures which may be reached during the rebound period probably indicate increased intensity of the disease. It has been further noted that with the induction of a complete remission the fibrinogen usually falls to within the normal range. In 27 courses of treatment it fell only once below the normal minimum of 190 milligrams per cent. On this occasion the fibrinogen reached a low value of 140 milligrams per cent. This behavior is in contrast to that of the euglobulin fraction. Not infrequently, the euglobulin may drop far below normal even with little clinical improvement, and here the response is more suggestive of hormonal suppression of protein synthesis. The fall in euglobulin is slower than that of fibrinogen and may continue after hormone administration has been stopped and a relapse is setting in. Certainly the euglobulin figures appear to be far less related to the activity of the arthritis. It is of interest to note in this connection, however, that both the total globulin and euglobulin may also ultimately rise to well above pretreatment levels. The significance of this is as yet far from clear.

SUMMARY

In rheumatoid arthritis, the sedimentation rate, as determined by the uncorrected Westergren technique, is a fair index of the level of the plasma fibrinogen, providing that the serum globulins are not markedly elevated and in the absence of severe anemia. Even when the globulin fractions are increased, a high sedimentation rate is usually associated with an elevated blood fibrinogen. The elevated fibrinogen content of the blood in turn appears to be an expression of the intensity of the disease process.

The administration of cortisone or ACTH in amounts sufficient to induce a clinical remission usually results in a dramatic fall in both the sedimentation rate and in the plasma fibrinogen, often to normal levels. During the relapse which follows the withdrawal of the hormones, both usually rise again. The plasma fibrinogen seems to continue to reflect the intensity of the disease during the period of these rapid changes, although the sedimentation rate may lag by 24 to 48 hours, resulting in a temporary disturbance in their interrelationship.

In the relapse or "rebound" period following abrupt cessation of hormone therapy the plasma fibrinogen was found frequently to rise not only to the pretreatment levels but to values which were as much as 50 per cent above those which existed before treatment. Since the sedimentation rate does not reflect fibrinogen concentrations above 700 milligrams per cent, it does not reveal the high rebound fibrinogen levels which may occur during the relapse period.

Changes in the serum globulins occur more slowly and present comparatively little relationship to the sedimentation rate or to the intensity of the arthritis. During the remissions induced by hormone therapy these serum proteins may fall well below their normal concentrations.

ACKNOWLEDGMENT

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THE USE OF K^{42} OR P^{32} LABELED ERYTHROCYTES AND I^{131} TAGGED HUMAN SERUM ALBUMIN IN SIMULTANEOUS BLOOD VOLUME DETERMINATIONS [1]

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Recent methods of measuring the volume of the circulating blood have employed the dilution principle, using either labeled red blood cells or substances which presumably travel with the plasma. Earlier methods made use of carbon monoxide (1) or specific agglutinogens (2) by the Ashby technique (3) as specific erythrocyte labels. In the past few years a variety of radioisotopes, Fe^{55}, Fe^{59} (4, 5), Cr^{51} (6), P^{32} (7), and K^{42} (8), have been found useful for this purpose. The most commonly employed plasma soluble substance has been Evans blue dye, T-1824 (9), its successful usefulness depending on its presumed rapid and complete binding to the circulating albumin (10). Recently plasma proteins tagged with I^{131} have been used in the determination of blood volume (11–13).

In a number of reports (3, 5, 14–18) simultaneous studies have yielded higher values for total blood volume by the dye method than by tagged erythrocyte methods. This finding led to the conclusion that the average body hematocrit is lower than the peripheral vessel hematocrit. Offered in support of this concept is the observation that post-phlebotomy red blood cell volumes calculated from the dye plasma volume and the hematocrit appeared lower than predicted from the pre-hemorrhage red cell value and the volume of red cells removed (19).

A contradictory opinion was presented by Gregersen (20) who found that blood volumes calculated from T-1824 dye dilution and the CO tagged red blood cell method gave values in satisfactory agreement and by Noble and Gregersen (21) who found that the increase in red blood cell volume after transfusion as determined from the dye plasma volume and hematocrit agreed well with the quantity of cells given in transfusion. Hopper, Tabor, and Winkler (22) also obtained almost identical results for average blood volume values calculated from T-1824 and CO tagged red blood cell dilution but concluded that neither method had been shown regularly to measure blood volume. Subsequently, Nickerson, Sharpe, Root, Fleming, and Gregersen (23) found that the radioactive iron (Fe^{55}) tagged erythrocyte method gave lower blood volumes than the T-1824 dye method.

A further disagreement was introduced by Mayerson, Lyons, Parson, Nieset, and Trautman (24) who found almost identical values in simultaneous studies utilizing Evans blue dye and P^{32} tagged red blood cells when they applied the 8½% hematocrit correction factor of Chapin and Ross (25) for trapped plasma. Using the same correction factor they recalculated the data of Gibson, Peacock, Seligman, and Sack (5) and Meneely, Wells, and Hahn (17) and obtained similar values for total blood volume as determined by the tagged red blood cell and Evans blue methods. They concluded that the appearance of differences between average body hematocrit and the peripheral vessel hematocrit arises chiefly from neglect of correction for trapped plasma in the centrifuged hematocrit tube. However, two recent similar studies (26, 27) demonstrated such differences even when the same correction factor was applied.

The demonstration that Evans blue dye appears in lymph fluid shortly after injection (28) and that dye concentration in the blood stream decreases at a relatively rapid rate during the first few minutes (29) has led to questions concerning the validity of its use as a measure of plasma volume with the implication that loss from the intravascular circulation leads to a significant overestimation (30, 31).

[1] Reviewed in the Veterans Administration and published with the approval of the Chief Medical Director. The statements and conclusions published by the authors are the results of their own study and do not necessarily reflect the opinion or policy of the Veterans Administration.

Crispell, Porter, and Nieset (13) found comparable results for plasma volumes obtained with Evans blue and with I^{131} tagged serum albumin. However, Storaasli, Krieger, Friedell, and Holden (12) found that the volume obtained with T-1824 was considerably greater than that with iodinated protein in all but one of 15 cases. On the other hand, simultaneous studies by this group of workers (11), using P^{32} tagged red blood cells and radio-iodinated serum protein in dogs, gave almost identical values. These latter results would imply that the higher blood volume values obtained with Evans blue as compared to tagged red blood cell methods are presumably in error due to overestimation by the former method.

In view of the contradictory evidence bearing on the relative validity of plasma soluble substances and tagged erythrocyte methods in blood volume determinations, it seemed advisable to reinvestigate this problem in a manner which would avoid points of previous dispute. In the present study simultaneous determinations of the blood volume of human subjects made with I^{131} labeled albumin and with P^{32} or K^{42} tagged red blood cells have been compared and the problem of the average body hematocrit has been considered.

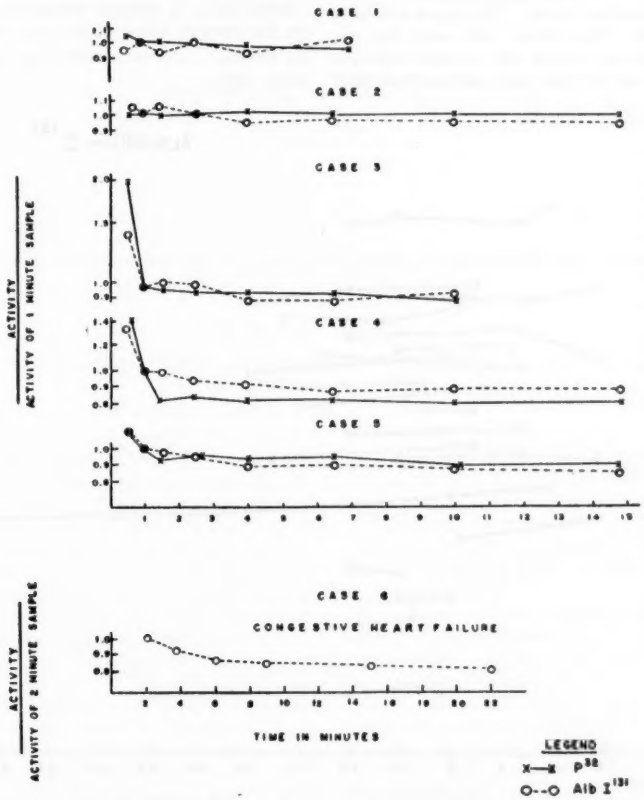

FIG. 1. SEMILOGARITHMIC PLOT—ARTERIAL CONCENTRATIONS OF P^{32} TAGGED RBC AND I^{131} TAGGED ALBUMIN DURING THE MIXING PERIOD

The concentrations are plotted relative to the one minute point in cases 1–5 and and to the two minute point in case 6.

The influence of errors of the centrifuged hematocrit value on the ratio of the peripheral vessel hematocrit to average body hematocrit has been obviated by the assay of whole blood rather than of plasma or red blood cells.

METHODS

Subjects were hospitalized patients of the Veterans Administration Hospital, Bronx, New York, and all were kept at complete bed rest for a minimum of three hours prior to the studies.

The K^{42} and P^{32} were obtained from Oak Ridge National Laboratory and were prepared for human use in neutral sterile isotonic saline solution. Ten ml. of heparinized blood were incubated under sterile conditions in a rubber-stoppered centrifuge tube at 37° for two hours with either 50 μc. carrier-free P^{32} or with 200 μc. K^{42} contained in 50 mg. stable potassium carrier. The plasma and buffy coat were removed. The labeled cells were then repeatedly centrifuged and washed with isotonic saline until the total activity in the final wash, performed no more than one-half hour prior to injection, was less than 1% of that activity bound to the red blood cells. Usually two to three washings were sufficient. The red blood cells were then prepared for injection by suspension in an equal volume of saline. Approximately 5 ml. of the suspension containing 7 to 12 μc. P^{32} or 2 to 4 μc. K^{42} were used for injection.

I^{131} labeled serum albumin was obtained from Abbott Laboratories and was prepared for injection of approximately 100 μc. in 5 ml. by dilution with sterile isotonic saline. Three to 4% of the radioactivity of earlier lots was found to be non-protein bound as determined by 24 to 48 hour dialysis and by trichloracetic acid precipitation. Seventy-five per cent of the total dialyzable activity appeared outside the membrane within the first hour. In all the arterial studies and in some other cases, prior to injection, dialysis under sterile conditions was performed against saline in beakers changed at frequent intervals. In the cases in which dialysis was not performed prior to injection, necessary corrections as described below were made.

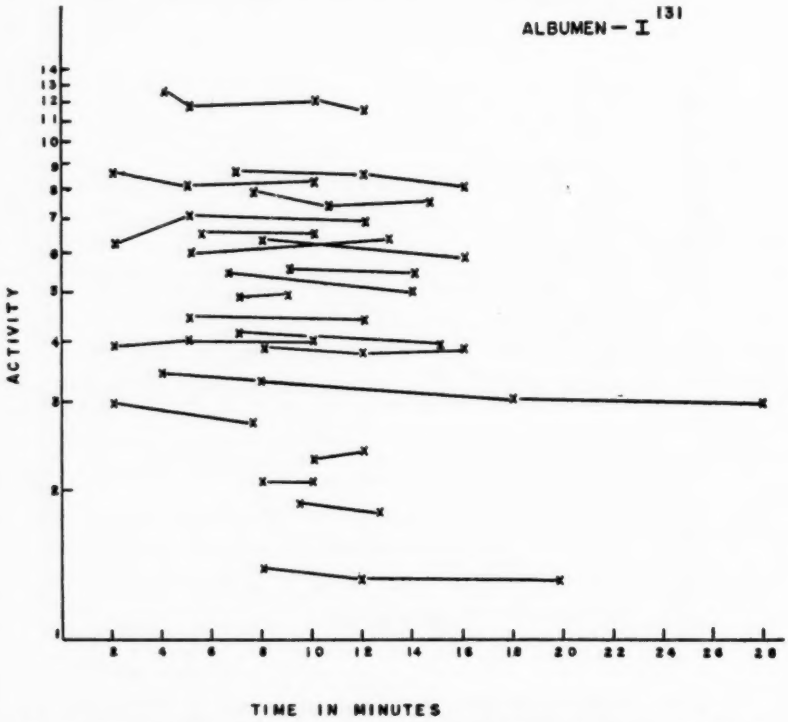

FIG. 2. SEMILOGARITHMIC PLOT—VARIATION OF VENOUS CONCENTRATIONS OF I^{131} TAGGED ALBUMIN WITH TIME

In each case activities are plotted relative to the first point.

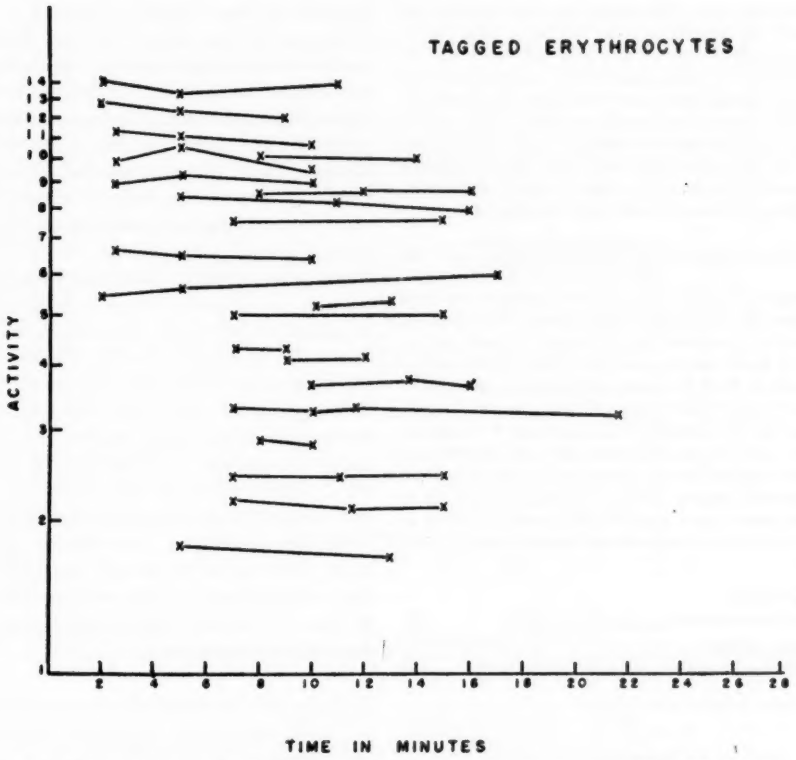

FIG. 3. SEMILOGARTHMIC PLOT—VARIATION OF VENOUS CONCENTRATIONS OF P^{32} OR K^{42} TAGGED ERYTHROCYTES WITH TIME

In each case activities are plotted relative to the first point.

Radioactivity of the injected albumin I^{131} solutions and of the withdrawn whole blood samples was determined by dilution of known volumes to 100 ml. in Marinelli beakers placed around an RCL bismuth walled Geiger counter. The sensitivity of this arrangement was 4,500 counts/min./μc. I^{131}. Since gamma counting was employed, the I^{131} could be assayed in the presence of P^{32}. When K^{42} labeled red blood cells were employed, this short lived isotope ($T\frac{1}{2} = 12.44$ hrs.) was allowed to decay for a suitable length of time before the I^{131} was assayed.

Radioactivity of the injected red blood cell suspensions in suitable dilutions and of the withdrawn whole blood specimens was assayed in a horizontal mount containing a thin walled tetramethyl lead filled beta-gamma counter. When 5 ml. liquid samples were placed in a fixed position under the counter, the sensitivity of the arrangement was 6.35×10^4 counts/min./μc. P^{32} and 1.63×10^5 counts/min./μc. K^{42}. No detectable differences in sensitivity due to unequal absorption by water and whole blood were found for either of the isotopes under the conditions specified.

All counting systems were shielded with 1 inch of lead. A minimum of 5,000 to 10,000 counts was recorded for each specimen. The counting rates of the samples were 20 to 50 times the background count.

Hematocrits were determined in Wintrobe tubes subjected to a maximum R.C.F. of 2,000 g at the tip for one-half hour.

For greater accuracy, weighed solutions were injected and the volumes calculated from the weight and measured density of these solutions. For determinations using venous samples, the syringes were weighed empty and with the appropriate solution. These were then rinsed several times with the patient's blood during the injection to insure complete delivery. For the arterial studies, where rapid delivery was desired, the syringe and needle were weighed with the solution to be injected and again after injection without rinsing, care being taken to prevent loss of residual solution within the needle at the time of withdrawal from the vein.

All injections were preceded by the aspiration and reinjection of 10 ml. of blood to insure central location of

the needle in the vein. The syringe was then removed and the tagged cell suspension was injected. After blood sampling from the opposite arm was completed, the albumin solution was then injected and sampling was repeated in a similar fashion at the same time intervals. For arterial samples an indwelling needle fitted with a stylus was inserted into the brachial artery prior to the injection of the tagged material. All blood specimens were taken without stasis in syringes wetted with heparin.

The following formulae were used in calculations:

$$\text{Total blood volume} = \frac{\text{Total injected activity}}{\text{Activity/ml. whole blood}}. \quad (1)$$

The average of the last two withdrawn samples was used to calculate the activity/ml. whole blood. The times of withdrawal of specimens for the tagged albumin or the tagged red blood samples were the same. Iodide is distributed within 10 to 15 minutes into a space equal to 20% of the body weight (32) compared to the usual 5% space which the plasma occupies. When albumin I^{131} solutions were used without prior dialysis the total injected activity was corrected for by subtracting from it 75% of the total dialyzable activity (3–4%) of that solution. It is clear that even a gross error in this correction factor is unlikely to affect the blood volume determination by more than 1%.

V_c = RBC volume
= Total blood volume$_{\text{tagged RBC}} \times$ hct. $\times 0.98$.[2] (2)

V_p = Plasma volume
= Total blood volume$_{\text{albumin } I^{131}} \times (1 - 0.98 \times \text{hct.})$. (3)

$$\text{Average body hematocrit} = \frac{V_c}{V_c + V_p}.$$

RESULTS

1) *Mixing time of erythrocytes and albumin*

To determine the relative rates of mixing of the albumin and the erythrocytes, multiple arterial samples of each were obtained from five subjects over the first seven to 15 minutes following injection, the initial sample being taken at 30 seconds. In one subject with congestive heart failure the mixing of the tagged albumin alone was studied. Simultaneous studies show that mixing of the tagged red blood cells and of the tagged albumin is approximately 90% complete within the first 60–90 seconds unless circulation is markedly impaired by heart failure (Figure 1). The rate of decrease of both tagged cells and the tagged albumin concentrations between one and one and a half minutes and seven to 10 minutes is in general higher than that noted at subsequent times. The possibility seems unlikely that excess early loss of albumin from the intravascular circulation occurred, in view of the similar changes which took place in the tagged red cell concentrations. The relatively rapid early fall in concentration must therefore be ascribed to continued mixing which appears to be virtually complete within five to 10 minutes after injection. The venous concentration curves for the tagged albumin (Figure 2) and for the tagged red blood cells (Figure 3) in the remaining subjects also show that the differences between the five to eight and the 12 to 25 minute concentrations are accountable by random variation. Other studies in this laboratory (34, 35) have shown that the rate of loss of activity from the circulation of either of these labeled substances is of the order of 1% or less per quarter hour during the first few hours. Thus for samples drawn at 10 and 15 minutes, extrapolated corrections to zero time are unnecessary.

2) *Comparison of blood volume determinations with tagged red blood cells and I^{131} tagged albumin*

The hematocrit values, whole blood volume data and calculated values for red blood cell volume (V_c), plasma volume (V_p), average body hematocrit (av. b. hct.) and the ratio of the average body hematocrit to the peripheral vessel hematocrit $\left(\frac{\text{av. b. hct.}}{\text{p. v. hct.}}\right)$ are given in Table I. In every case the blood volume determined by dilution of the tagged albumin was greater than that obtained from the tagged erythrocyte studies. Accordingly, the values for whole blood volume calculated by adding the albumin I^{131} plasma volume (V_p) and the tagged erythrocyte volume (V_c) fall between the two. The average body hematocrit $\left(\frac{V_c}{V_c + V_p}\right)$ is therefore in all cases lower than the hematocrit in the large vessels. The mean value for the ratio of average body hematocrit/peripheral vessel hematocrit is 0.924 with a range from 0.860 to 0.996.

[2] Experiments in this laboratory involving the assay of the relative quantity of albumin labeled with I^{131} in the red blood cell column of the centrifuge tube as compared with the supernatant plasma have shown that under the conditions of centrifugation specified the hematocrit reading is in error by not more than 2% (33). Therefore, the correction factor of 0.98 has been applied to the hematocrit readings.

TABLE I

Summary of blood volume data in 25 studies on 21 subjects

Case No.	Diagnosis	Hct.	Corr'd hct.	P^{32} or K^{42} B.V.	V_e	Alb. I^{131} B.V.	V_p	$V_e + V_p$	$\frac{V_e}{V_e + V_p}$	Ven. $\frac{\text{Body hct.}}{\text{hct. corr'd}}$
1	Leukemia	36.5	35.8	P 5,900	2,100	6,040	3,880	5,990	35.2	.983
2	Sarcoidosis	47.1	46.2	P 4,400	2,030	4,935	2,660	4,690	43.3	.940
3	Polycythemia vera	65.2	63.7	P 4,170	3,000	5,330	1,950	4,950	60.5	.952
4	Sarcoidosis	51.0	50.0	P 5,780	2,890	6,080	3,040	5,930	48.75	.975
5	Normal	44.5	43.6	P 5,120	2,235	5,550	3,120	5,355	41.6	.955
6*a*	Polycythemia vera, ASHD; CHF	73.0	71.5	K 6,400	4,575	7,850	2,240	6,815	66.8	.935
*b**		34.0	33.3	P 4,950	1,650	5,990	3,990	5,640	29.3	.880
7	Cor pulmonale, CHF	44.0	43.1	K 6,600	2,840	7,700	4,390	7,230	39.3	.915
8	ASHD; CHF	38.0	37.2	P 4,000	1,490	4,930	3,090	4,580	32.5	.875
9*a*	Polycythemia vera	69.0	67.6	K 6,550	4,435	9,350	3,120	7,555	58.5	.865
b		67.0	65.6	P 7,240	4,750	8,570	2,950	7,900	60.1	.915
10*a*	Polycythemia vera	65.0	63.4	K 4,900	3,100	5,850	2,140	5,240	59.2	.934
*b**		43.0	42.1	P 4,800	2,020	5,880	3,410	5,430	37.1	.885
11	Pernicious anemia	16.5	16.2	P 4,660	750	4,670	3,915	4,665	16.1	.996
12	ASHD; CHF	47.1	46.2	P 4,460	2,060	5,790	3,120	5,180	39.7	.860
13	HASHD; CHF	45.5	44.6	P 5,800	2,580	7,060	3,920	6,500	39.7	.890
14	Pulmonary TB.	38.2	37.2	P 2,650	985	3,320	2,090	3,075	32.1	.860
15	Pulmonary berrylosis	50.3	49.3	P 4,440	2,175	4,780	2,420	4,590	47.3	.960
16*a*	Laennec's cirrhosis	40.0	39.2	P 6,170	2,415	6,750	4,100	6,515	37.0	.943
b†		38.0	37.2	P 6,295	2,330	7,120	4,470	6,700	34.9	.938
17	Laennec's cirrhosis	39.3	38.5	P 5,040	1,940	5,230	3,210	5,150	37.7	.977
18	Splenic vein thrombosis	37.5	36.7	P 5,140	1,880	6,040	3,820	5,700	32.9	.895
19	Laennec's cirrhosis	30.0	29.4	P 5,950	1,750	6,930	4,880	6,630	26.4	.897
20	Normal	36.8	36.1	P 4,590	1,660	4,950	3,160	4,820	34.3	.950
21	Polycythemia vera	61.9	60.7	P 6,590	3,990	7,950	3,140	7,130	56.0	.922

* After phlebotomy.
† After intravenous albumin.
ASHD—Arteriosclerotic heart disease.
CHF—Congestive heart failure.
HASHD—Hypertensive and arteriosclerotic heart disease.

3) One-half hour urinary radioactivity

When dialyzed albumin was used, the one-half hour urine specimens contained less than 0.1% of the injected activity. When undialyzed solutions containing 3–4% non-protein bound activity were injected, less than 0.25% of the injected activity was excreted in the urine during the half hour period following injection. Previous studies have shown that 5–10% of injected KI^{131} appears in the urine during this time (32).

DISCUSSION

The relationship between the plasma/erythrocyte ratios in large and small vessels has been a point of considerable dispute. An average body hematocrit lower than the peripheral vessel hematocrit has been interpreted as indicating that the plasma/red cell ratio in minute vessels throughout the body is greater than the ratio found in the large peripheral vessels which are readily available for sampling. A principal source of disagreement among previous workers arises in the method of estimating plasma volume by assay of plasma alone for dye content or radioactivity and of red blood cell volume by the assay of whole blood or packed erythrocytes. The accuracy of the hematocrit reading then becomes an important factor in the determination of the ratio of average body hematocrit to peripheral vessel hematocrit. Estimations of the error of the hematocrit have ranged from -5% to $+8.5\%$ (33). Thus, identical data for erythrocyte and plasma dilution may be obtained by different investigators, but, owing to the application of different correction factors for the same hematocrit, or of an identical correction for hematocrits with different errors, divergent interpretations are expressed. In most reports, the conditions of centrifugation under which the hematocrit readings were obtained are not given. It has been shown that the error of the hematocrit obtained in tubes spun for 30 minutes at 500 g is 8.5% but at 2,000 g is not more than 2% (33). From these considerations, it appears likely that discrepancies in previously reported studies may at times be related to calculations involved in the determination of total blood volume from the dye

plasma volume and the hematocrit rather than to real differences in the dilution factors.

In the present study this difficulty has been circumvented by assaying whole blood for tagged red cell and tagged albumin activity alike. Hence, the correction applied to the hematocrit reading is of negligible importance in evaluating the average body hematocrit/peripheral vessel hematocrit ratio because such a correction affects both to almost the same extent. In the present study this ratio was always less than one. However, its mean value is not as low as that obtained by some other workers using Evans blue and tagged cell methods, which again raises the possibility that the dye method leads to overestimation of plasma volume. Even if it is accepted that, when injected in proper concentration, most of the dye becomes bound to serum albumin, early loss from the intravascular circulation before complete binding has occurred cannot be excluded. The studies of Barnes, Loutit, and Reeve (36) *in vitro* have shown that the binding is not complete until 40 to 50 seconds at body temperature. The circulation time to the lungs is of the order of four to eight seconds and it is probable that some unbound fraction still remains at that time to pass through the pulmonary capillaries, or that the vascular endothelium may compete with the serum albumin for the binding of the dye. Meneely, Wells, and Hahn (17) showed that, following injection of dye labeled plasma from a donor, the "mixing phase" was less marked than that following the administration of dye alone. The staining of veins subjected to numerous injections of the dye is obvious even through the skin (37). Tagged albumin is free of these objections, and would seem to be more satisfactory for estimation of plasma volume than a dye, the suitability of which depends upon its becoming bound to albumin.

It has been stated that the time for mixing of plasma soluble substances and red blood cells is perceptibly different, the latter being the more rapid (15). No consistent differences were observed after 30 seconds in the five cases studied here. In the case of both, mixing to the extent of about 90% of the equilibrium value took place within one to one and a half minutes but the attainment of apparent equilibrium took five to 10 minutes. This suggests that most of the circulating blood is in very free intercommunication but that there are some depots to the extent of 5 to 10% of the total blood volume which are less readily penetrated. The situation in shock has not been investigated. In the case of circulatory impairment, as in heart failure, the earlier phase of mixing may be retarded but equilibrium was fairly well established in less than 10 minutes in the one case studied. Since, after the initial mixing period of five to 10 minutes, the rate of fall in the concentration of the tagged red blood cells or albumin is less than 1% per quarter hour, samples drawn between 10 and 20 minutes after injection do not require an extrapolation correction.

SUMMARY

1. Simultaneous blood volume determinations utilizing albumin tagged with I^{131} and erythrocytes tagged with P^{32} or K^{42} have been performed in 25 tests on 21 subjects. In every instance, the whole blood volume evaluated by the tagged albumin dilution was greater than that derived from the dilution of the tagged erythrocytes. The average body hematocrit/peripheral vessel hematocrit ranged from 0.860 to 0.996 with a mean value of 0.924. Since whole blood samples were assayed, these ratios did not depend on the accuracy of the observed hematocrit reading.

2. Arterial mixing curves revealed that apparent equilibrium may not be attained until five to 10 minutes after injection although the concentrations at one to one and a half minutes are only approximately 10% above the equilibrium values. There is no essential difference in the mixing times of tagged albumin and erythrocytes.

3. Albumin tagged with I^{131} is a satisfactory material for the estimation of circulating plasma volume. Owing to the difference between average body hematocrit and peripheral vessel hematocrit the accurate evaluation of whole blood volume requires independent determinations of plasma and red blood cell volumes.

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THE BIOLOGICAL DECAY CURVE OF P^{32} TAGGED ERYTHROCYTES. APPLICATION TO THE STUDY OF ACUTE CHANGES IN BLOOD VOLUME [1]

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Changes in circulating blood volume occurring over prolonged periods may be estimated by serial blood volume determinations. Employing the dilution principle, plasma soluble dyes (1, 2), serum proteins tagged with I^{131} (3–6) or isotope labeled erythrocytes (7–10) have been found useful for this purpose. For short term studies, however, the reinjection of these agents and their resampling involve considerable effort and lead to multiplication of errors, especially when many determinations are desired over a period of several hours. Because of the difficulties entailed in such studies, rapid alterations in blood volume have been evaluated from changes in the hematocrit values, hemoglobin concentration, red cell count, protein concentration, colloid osmotic pressure, plasma specific gravity, or some combination of these. Some of these parameters do not lend themselves to very precise quantitative assay and prove unsatisfactory as measures of small fluctuations in blood volume. Furthermore, changes in protein concentration, colloid osmotic pressure, or specific gravity may be unreliable for the evaluation of plasma volume variations because of the error introduced by the relatively rapid passage of albumin into extravascular spaces in the establishment of new equilibria. In addition, non-isooncotic expansion or contraction of blood volume may lead to changes in the size of the erythrocytes (11), thus introducing an error in the hematocrit value. Lyons, Avery and Jacobson (12) have emphasized that variations in hematocrit readings and serum protein concentrations fail to reflect quantitatively changes in plasma volume.

The present report is concerned with the concentration-time curves of circulating radioactivity over periods of hours to days following the injection of P^{32} tagged red blood cells and the application of such curves to the continuous study of fluctuations in blood volume in certain acute clinical and experimental situations.

METHODS

Subjects were hospitalized patients of the Veterans Administration Hospital, Bronx, N. Y., who were kept at complete bed rest for the control studies except where otherwise indicated. For the experiments lasting longer than 24 hours, patients were maintained at bed rest for a week because of the progressive hemoconcentration which occurs during the first few days in bed (13). In the experiments in which the effects of diuretics and digitalis were studied the patients were maintained on constant fluid intake throughout the control and experimental periods and on a low salt diet (less than 0.20 gm. Na/day) for a week or more prior to and during the study. The patients' clinical diagnoses are given in Table I.

The methods used for the determination of the initial blood volumes and hematocrit values and for the assay of radioactivity of the whole blood samples were identical with those previously reported (6). Hemoglobin concentration was determined by the method of Wong (14) with the Leitz colorimeter. All blood samples were drawn without tourniquet stasis into syringes moistened with heparin. Venipuncture was performed in the arm contralateral to the site of injection. Sampling was begun 12 minutes after the injection to allow for adequate mixing (6).

The P^{32} concentrations in whole blood, corrected for physical decay ($T_{\frac{1}{2}}$ = 14.3 days), were plotted on semilogarithmic paper as a function of time and the best straight line was drawn through the points obtained during the control period. The biological half life[2] was determined directly from this line. From the extrapolation of this line into the experimental period, the expected concentration at any time could be determined.

[1] Reviewed in the Veterans Administration and published with the approval of the Chief Medical Director. The statements and conclusions published by the authors are the results of their own study and do not necessarily reflect the opinion or policy of the Veterans Administration.

[2] In this report the biological half life is defined as the time required for the radioactivity concentration in whole blood to fall to 50% of the initial activity after correction for physical decay.

TABLE I

Summary of data on blood volume determinations in 18 subjects

Experimental condition	Case No.	Initial volumes (*ml.*)			Duration control period (*hrs.*)	Biological half life (*hrs.*)	Hours following onset of experimental condition	New volumes (*ml.*)			Absolute (*ml.*) and % change			Diagnosis and comments
		T.B.V.	RBC V.	P.V.				T.B.V.	RBC V.	P.V.	T.B.V.	RBC V.	P.V.	
Bed rest—control	1	6,050	3,190	2,860	70	36.4								Polycythemia vera. Three 500 ml. phlebotomies during week prior to study
	2	4,700	1,185	3,515	70	23.5								Pernicious anemia. Intravenous vitamin B_{12} given on first day of observations
I.v. saline infusion, 500 ml.	3	5,590	1,560	4,030	4.9	12.6	0–1.6	no detectable change						Chronic glomerulonephritis, nephrotic stage
I.v. human serum albumin, 50 gm.	4	6,690	2,430	4,260	23.5	25.2	4	8,390	hct. not obtained		+1,700 +25.4%			Laennec's cirrhosis, compensated. 80 ml. RBC removed during study
							26	6,950	2,380	4,570	+310 +4.65%	−50 +1.2%*	+325 +7.5%	
							reinjection study 26.3	6,800	2,330	4,470				
Phlebotomy, 650 ml.	5	4,800	2,310	2,490	3.5	21.8	1.7	5,130	2,310	2,820	+330 +6.9%	0 +14.5%*	+330 +13.2%	Cor pulmonale, 335 ml. RBC removed in phlebotomy
Phlebotomy, 625 ml.	6	7,860	4,200	3,660	23.5	19.3	1.5	8,380	hct. not obtained		+520 +6.6%			Polycythemia vera
Paracentesis	7	5,700	1,620	4,080	3.0	19.0	1.5	5,250	1,560	3,690	−450 −7.9%	−60 −3.7%	−390 −9.6%	Laennec's cirrhosis, decompensated
I.v. mercurial diuretic (Mercupurin)	8	4,880	1,920	2,960	2.8	16.9	5.0	4,450	1,850	2,600	−430 −8.8%	−70 −3.7%	−360 −12.2%	Hypertensive and arteriosclerotic heart disease; congestive heart failure. 5 hr. urine excretion, 640 ml. in excess of similar control period
							12.0	4,240	hct. not obtained		−640 −13.1%			
	9	6,310	3,280	3,030	2.5	22.5	22.0	4,760	hct. not obtained		−1,550 −24.6%			Hypertensive and arteriosclerotic heart disease; congestive heart failure. 22 hr. urine excretion, 4,100 ml. in excess of similar control period

* Volume of RBC withdrawn taken into account.
T.B.V.—total blood volume.
RBC V.—red blood cell volume.
P.V.—plasma volume.

TABLE I—*Continued*

Experimental condition	Case No.	Initial volumes (*ml.*)			Duration control period (*hrs.*)	Biological half life (*hrs.*)	Hours following onset of experimental condition	New volumes (*ml.*)			Absolute (*ml.*) and % change			Diagnosis and comments	
		T.B.V.	RBC V.	P.V.				T.B.V.	RBC V.	P.V.	T.B.V.	RBC V.	P.V.		
Effect of ambulation and recumbency	10	7,430	2,860	4,570	23.5	23.0	amb. 3.0	7,070	2,840	4,230	−360 −4.8%	−20 −0.7%	−340 −7.4%	Hypertensive and arteriosclerotic heart disease; congestive heart failure	Control period at bed rest. Followed by ambulation. Followed by return to bed
							bed 1.6	8,350	3,200	5,150	+920 +12.4%	+340 +11.9%	+580 +12.7%		
	11	4,340	1,610	2,730	3.0	25.0	amb. 3.0	4,140	1,590	2,550	−200 −4.7%	−20 −1.2%	−180 −6.6%		
							bed 2.0	4,850	1,650	3,200	+510 +11.8%	+40 +2.5%	+470 +17.1%		
	12	6,550	3,110	3,440	3.0	25.6	amb. 2.0	6,450	3,040	3,410	−100 −1.5%	−70 −2.3%	−30 −0.9%		
							bed 2.0	6,970	3,220	3,750	+420 +6.3%	+110 +3.5%	+310 +9.0%		
	13	5,680	2,240	3,440	23.0	21.2	amb. 3.8	4,740	2,040	2,700	−940 −16.5%	−200 −8.9%	−740 −21.5%	Convalescent rheumatic arthritis. No heart disease	
							bed 2.0	5,000	2,120	2,880	−680 −12.0%	−120 −5.4%	−560 −16.3%		
	14	7,000	3,060	3,940	30.0	17.0	amb. 2.0 bed 2.0	no detectable change						Glaucoma. No heart disease	
Effect of recumbency	15	5,700	2,800	2,900	2.5	39.0	bed 1.0	6,400	3,070	3,330	+700 +12.3%	+270 +9.6%	+430 +14.8%	Hypertensive and arteriosclerotic heart disease; congestive heart failure	Control period during ambulation. Followed by return to bed
	16	5,530	2,130	3,400	2.5	12.0	bed 1.0	5,950	2,050	3,900	+420 +7.6%	−80 −3.8%	+500 +14.7%		
Effect of digitalis in heart failure	17	4,720	2,060	2,660	26.0	34.2	20	4,120	1,880	2,240	−600 −12.7%	−180 −8.7%	−420 −15.8%	Hypertensive and arteriosclerotic heart disease, congestive heart failure; 20 hr. urine excretion, 1,500 ml. in excess of similar control period	
	18	6,650	2,540	4,110	22.0	21.0	22	5,640	2,230	3,410	−1,010 −15.2%	−320 −12.6%	−700 −17%	Hypertensive and arteriosclerotic heart disease; congestive heart failure. 36 hr. urine excretion, 1,070 ml. in excess of similar control period	
							34†	5,200	2,120	3,080	−1,450 −21.8%	−420 −16.6%	−1,030 −25.1%		

† Values probably quantitatively unreliable for reasons discussed in text.

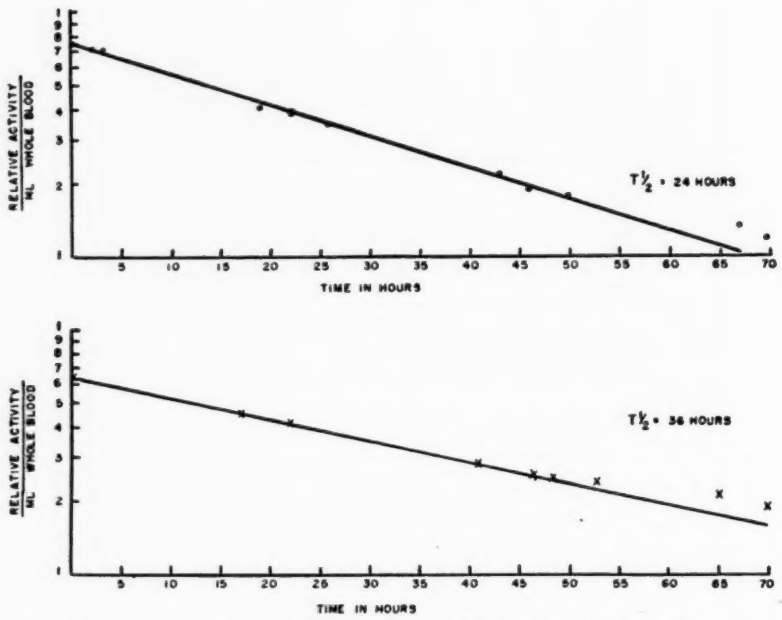

FIG. 1. SEMILOGARITHMIC PLOTS OF RELATIVE ACTIVITY/ML. WHOLE BLOOD AS A FUNCTION OF TIME IN SUBJECTS AT BED REST

Lower curve, case 1; upper curve, case 2.

The following formulae were used in calculating the initial volumes:

$$\text{RBC volume}_{\text{initial}} = \frac{\text{Injected activity}}{\text{Average activity of 12–15 min. whole blood samples}} \times \text{observed hct.} \times 0.98.^{3} \quad (1)$$

The mean value of the ratio average body hematocrit/peripheral vessel hematocrit has been found to be 0.924 (6). Therefore

$$\text{Av. body hct.} = 0.924 \times \text{observed hct.} \times 0.98, \quad (2)$$

$$\text{Total blood volume}_{\text{initial}} = \frac{\text{RBC volume}}{\text{Av. body hct.}}, \quad (3)$$

$$\text{Plasma volume}_{\text{initial}} = \text{Total blood volume}_{\text{initial}} - \text{RBC volume}_{\text{initial}}. \quad (4)$$

Following hemodilution or hemoconcentration, the new volumes were calculated as follows:

$$\text{Total blood volume}_{\text{new}} = \text{Total blood volume}_{\text{initial}} \times \frac{\text{expected activity}}{\text{observed activity}}. \quad (5)$$

[3] The factor 0.98 is used to correct for plasma trapped in the erythrocyte column which under the conditions of centrifugation used in this study has been found to be 2% (15).

The expected activity was obtained by extrapolation of the control decay curve to the same time as the observed activity.

$$\text{RBC volume}_{\text{new}} = \text{Total blood volume}_{\text{new}} \times \text{av. body hct.}_{\text{new}}, \quad (6)$$

$$\text{Plasma volume}_{\text{new}} = \text{Total blood volume}_{\text{new}} - \text{RBC volume}_{\text{new}}. \quad (7)$$

It is appreciated that the figure 0.924 represents only an average value for the ratio of average body hematocrit/peripheral vessel hematocrit and further, that this ratio may change during the course of the experiment. However, the use of this value represents the best compromise short of doing repeated erythrocyte and plasma volume determinations at every point.

RESULTS

I. Biological decay curves of circulating radioactivity following injections of P^{32} tagged red blood cells in subjects in the "steady" state

In two subjects at bed rest the curves were followed for 70 hours or for two and three half lives, respectively (Figure 1). When the radioactivity concentrations were plotted on semi-logarithmic

paper as a function of time, a straight line could be drawn through the points obtained during the first 50 hours in both cases. Subsequently the points fell above the straight line with gradually increasing differences. This departure from the simple exponential relationship after several days is in part explained by the increasing significance of the small amount of P^{32} in the plasma compared to the diminishing activity still remaining in the cells and the activity which has returned to the blood stream in newly formed cells. The two cases selected for these studies were chosen to exaggerate this latter factor (polycythemia vera recently subjected to repeated phlebotomy and pernicious anemia under treatment with vitamin B_{12}).

Table I gives the biological half lives observed in 18 subjects. It also includes the data of experiments in which acute changes in blood volume were induced. In these 18 experiments the biological half life varied from 12.0 to 39.0 hours. The reasons for this variation have not been investigated but may be related to the differences in the rate of phosphate transfer. In nine cases, the control observations were continued for 22 hours or longer and in the other nine cases for 2.5 to 4.9 hours. In all of these cases the observed points deviated from the straight line by no more than 2–3%. The longer the desired period of experimental observations, the longer is the required control period. This is essential in order to minimize the effect of slight errors in the slope of the exponential decay line and to increase the significance of deviations of the experimental points from the expected values.

II. Application of the biological decay curves to the evaluation of changes in blood volume

Figure 2 (A)–(G) shows the changes in blood volume occurring in a number of different situations. In each case the extrapolation of the control curve as a discontinuous line into the experi-

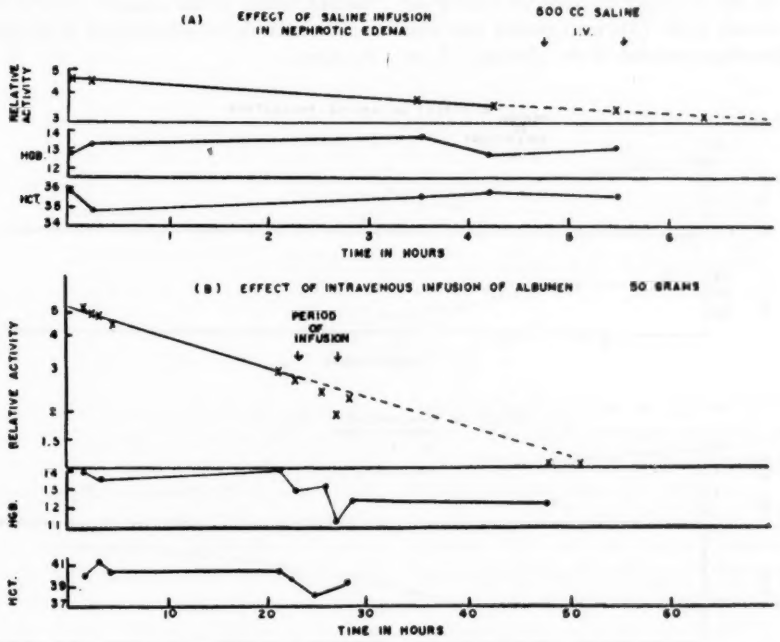

FIG. 2(A)–(G). SEMILOGARITHMIC PLOTS OF RELATIVE ACTIVITY/ML. WHOLE BLOOD AS A FUNCTION OF TIME

The points joined by the solid lines were obtained during the control period.

mental period is used to calculate the magnitude of the changes resulting. Where points fall above the line, hemoconcentration and decrease in total blood volume have occurred. The reverse is true for points falling below the line.

(*A*) *The effect of a 500 ml. intravenous saline infusion in a patient with marked nephrotic edema* (*Figure 2A*). The absence of detectable hemodilution demonstrates the rapidity with which the saline solution left the intravascular circulation. As a conservative estimate, this patient had 30 liters of extracellular fluid. Thus, if proportional distribution of the infused saline had taken place in plasma, interstitial, and edema fluid, a change in total blood volume of less than 1% would have been expected.

(*B*) *The effect of an intravenous infusion of 50 gm. of salt-free albumin in 200 ml. of solution in a patient with compensated cirrhosis of the liver* (*Figure 2B*). The marked hemodilution due to augmentation of plasma volume as well as the rapid, though only partial, restoration is clearly evident. By the following morning the total blood volume was only 4.6% (310 ml.) greater than that prior to the administration of the albumin. A reinjection study was performed at this time and the results agreed well with the calculated values (Table I). The equilibration of intravascular with extravascular albumin is dealt with elsewhere (16).

(*C*) The effect of phlebotomy (*Figure 2C*). Two cases have been studied in this manner. Both showed identical changes. The curve of one of these (case 5) is shown. Rapid hemodilution which resulted in a total blood volume exceeding the original occurred within one and one-half hours following the phlebotomy in both cases.

(*D*) *Effect of paracentesis in a patient with decompensated cirrhosis of the liver* (*Figure 2D*). The rapid hemoconcentration, followed by a tendency toward restoration of the original volume, is suggestive of a markedly accelerated loss of plasma into the peritoneal cavity. This loss apparently progressed more rapidly at first than the ability of the extravascular fluid accumulation to restore plasma volume. When more prolonged, this reduction of plasma volume may be a significant factor in the relative oliguria frequently noted on the day of paracentesis in this patient and in others.

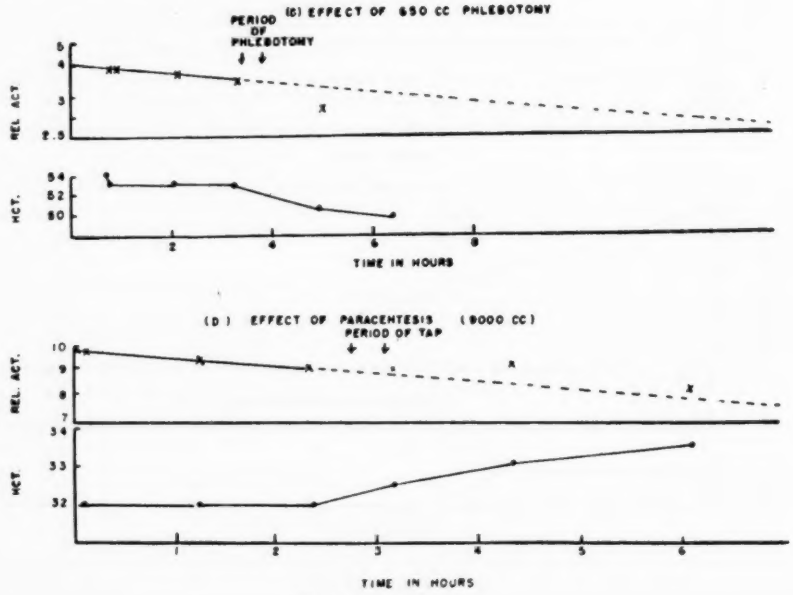

FIG. 2. (*Continued.*)

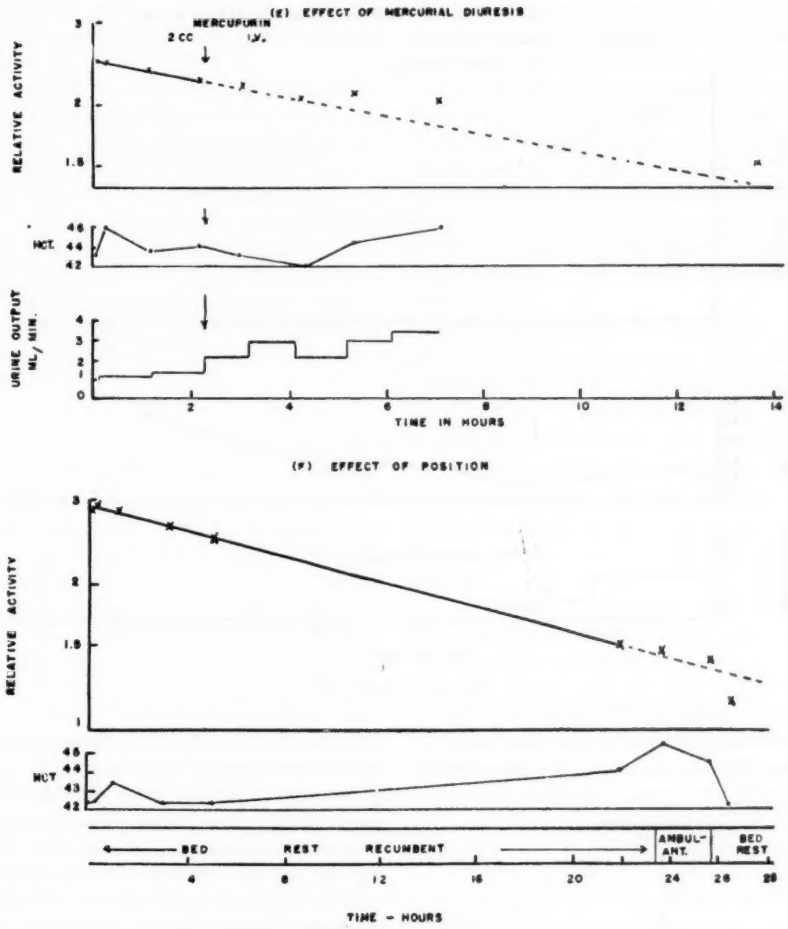

FIG. 2. (*Continued.*)

(*E*) *Effect of mercurial diuresis* (*Figure 2E*). Two patients with marked edema due to heart failure were studied following the intravenous administration of 2 ml. of Mercupurin. In one (case 8, Figure 2E), there appeared to be an insignificant fall in the hematocrit shortly after onset of diuresis. In both cases the P^{32} curves demonstrated progressive hemoconcentration beginning within a few hours after the onset of diuresis. This evidence militates against any hemodilution prior to diuresis. Here too, then, the ability of extracellular fluid stores to restore plasma volume lagged behind the loss of plasma water into the urine. The plasma volume remained appreciably reduced even by 12 and 22 hours, respectively, following the injections.

(*F*) *Effect of position* (*Figure 2F*). Three patients with slight dependent edema and suffering from attacks of paroxysmal nocturnal dyspnea due to heart failure, and two subjects with no evidence of heart disease were studied following control observations at bed rest. A typical curve of the cardiac cases is shown in Figure 2F. In all of the cardiac patients there was noted some de-

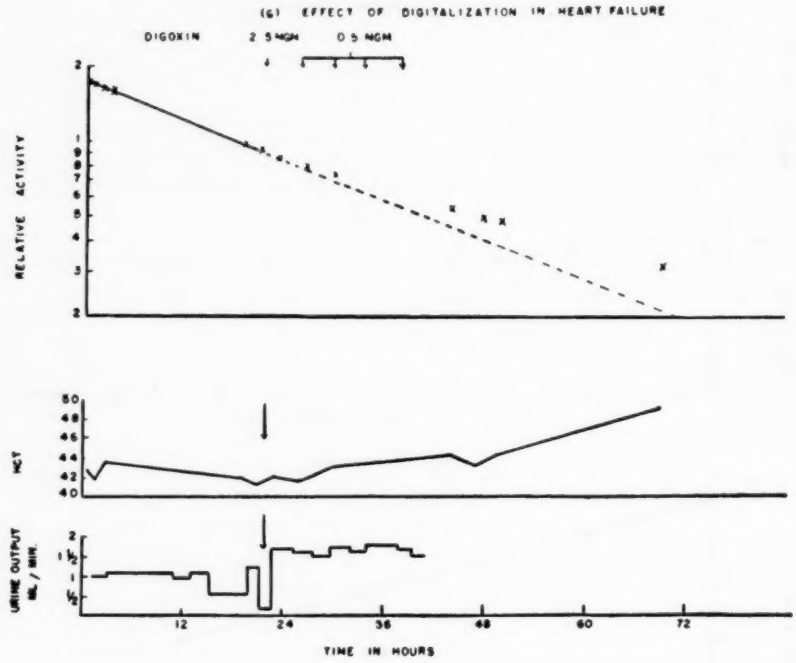

FIG. 2. (*Continued.*)

gree of hemoconcentration during the period of ambulation following bed rest. The hemodilution on return to recumbency was in excess of that required to restore the original volume. However, in both the noncardiac cases, this latter phenomenon was not observed even though, in one, significant hemoconcentration had taken place during the ambulant period. In two other patients with heart failure (cases 15 and 16), the control curve was obtained during ambulation. Assumption of the recumbent position led to significant hemodilution.

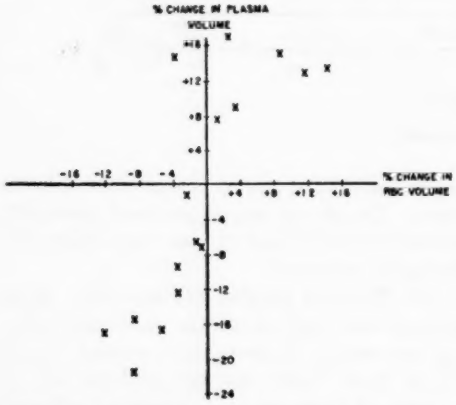

FIG. 3. THE EFFECT OF CHANGES IN PLASMA VOLUME (RESULTING FROM CONCENTRATION OR DILUTION) ON ERYTHROCYTE SIZE, AS DETERMINED BY CHANGES IN TOTAL RBC VOLUME

(*G*) *Effect of digitalis in heart failure* (*hypertensive and arteriosclerotic disease with normal sinus rhythm*) (*Figure 2G*). The findings were identical in both cases given Digoxin. There was no detectable change in blood volume until several hours after the onset of diuresis. After this time there occurred progressive hemoconcentration resembling the pattern observed following mercurial diuresis. The position of the 72 hour point in case 18 (Figure 2G) is undoubtedly too high, in light of the observations presented in Figure 1. The accompanying change in hem-

atocrit, however, leaves little doubt as to the marked hemoconcentration which had occurred at this time.

Figure 3 shows the effect of changes in plasma volume on the total red blood cell volume. It is of interest to note that increase and decrease in total erythrocyte mass associated with hemodilution and hemoconcentration, respectively, were observed in all cases but one (case 16) where hematocrit values were available for calculation. In general these changes were more marked when plasma volume alterations were greatest. In many cases, however, these changes were so slight as to be within the range of technical error.

DISCUSSION

Significant mobilizable blood depots, restricted from the general circulation, have not been demonstrated in man (17). Acute variations in blood volume, exclusive of those due to hemorrhage or transfusion, therefore, have been considered chiefly dependent on changes in the size of the plasma compartment. In the "steady" state at bed rest, the plasma volume appears to remain remarkably constant, aside from the slow and gradual hemoconcentration which occurs during the first few days as demonstrated by Widdowson and McCance (13). Therefore, experiments requiring observations over more than 24 hours should be preceded by three to four days of bed rest.

Metcalf (11) has observed, following serum transfusions, a striking correlation between the per cent increase in protein concentration and per cent decrease in the size of the red blood cells, leading to marked changes in hematocrit values independent of the effect of plasma volume shifts. However, consistent changes in the size of the red cells have not been observed by another group (18) after the infusion of hyperoncotic albumin solutions. In the present study, hemodilution and hemoconcentration appeared to exert slight effects on the size of the red cells, assuming that erythrocytes did not leave or enter the active circulation during the period of the observations. On the assumption that total circulating hemoglobin remains unchanged, relative changes in plasma volume may be evaluated by calculation from simultaneously observed changes in hematocrit and hemoglobin concentration (18, 19), regardless of effects on erythrocyte size. For evaluation of absolute changes this method requires, in addition, a plasma volume determination. The method presented here allows for more precise quantitation of these changes in a simple manner, and its validity is not affected by mobilization of red blood cells from depots if such does occur.

While a thorough consideration of the physiological basis of the changes observed in the experiments reported here is not within the scope of this report, certain of the results deserve brief comment.

Calvin, Decherd, and Herrmann (20) and Stewart (21) noted early increases in plasma volume following administration of digitalis to patients in heart failure. The late effects were in reverse. In the two cases studied here, hemodilution was not noted at any time and diuresis was followed by hemoconcentration. Eichna and his associates (22) also failed to note significant changes in plasma volume prior to the diuresis produced by Digoxin.

In the cases of mercurial administration, the observation that diuresis occurs first and is followed by hemoconcentration is in accord with reports by others (23, 24, 12). The discordant finding of de Vries (25) that these changes occur only in nonedematous subjects is not borne out by the observations presented here. The absence of any change in the cases studied by Blumgart and his coworkers (26) may be discounted since in five of the 10 experiments, the excess urine loss amounted to less than 250 ml., and in no case did it exceed 1,000 ml. The present study gives no support to the concept promoted by some authors (20, 27) that a tissue effect causing a shift of fluid into the vascular system, with resulting hemodilution, precedes the diuresis produced by mercurial diuretics.

The effect of recumbency in increasing plasma volume in patients with congestive heart failure confirms the findings of Perera and Berliner (28). However, the overdilution, on return to bed, in excess of the previous hemoconcentration produced by ambulation, which was observed in all cardiac cases, has not been previously noted.

The rapid decrease of the plasma volume following its initial augmentation resulting from intravenous albumin administration is evidence of

the rapid disappearance of much of the added albumin from the circulation. Further observations and considerations of this problem are to be presented elsewhere (16).

SUMMARY AND CONCLUSIONS

1. Following intravenous administration of P^{32} labeled erythrocytes, a semilogarithmic plot of the radioactivity per unit volume of whole blood as a function of time is a straight line for at least 48 hours in subjects with unchanging blood volumes. The biological half lives in 18 experiments ranged from 12 to 39 hours.

2. This exponential relationship permits the determination of serial whole blood and plasma volumes with an error of 2–3%. It may therefore be used to quantitate rapid changes in these volumes without requiring reinjection techniques.

3. The observed alterations in blood volume produced by digitalis, mercurial diuretics, positional changes and human serum albumin infusion are briefly discussed.

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A METABOLIC STUDY OF A DIABETIC PATIENT: THE EFFECT OF VARIATIONS IN THE DOSAGE OF INSULIN UPON ADRENAL CORTICAL ACTIVITY AND UPON WATER, ELECTROLYTE AND NITROGEN EXCRETION [1]

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That alterations in adrenal cortical structure and function may accompany diabetes mellitus is indicated by a number of observations. Untreated alloxan-diabetic rats, for example, exhibit both gross (1) and microscopic (2) evidence of adrenal cortical hypertrophy, suggesting that adrenal cortical hyperfunction may accompany diabetes, at least in the absence of insulin therapy. Diabetic patients, on the other hand, tend to excrete 17-ketosteroids (3) and perhaps corticosteroids (4) at a lowered rate, and to react to surgical trauma with abnormally transient eosinopenia (5), suggesting that under certain circumstances there may be hypofunction, and possibly partial exhaustion, of the adrenal cortex.

Of the various metabolic abnormalities which might produce hyperfunction and ultimate attrition of the adrenal cortex in diabetic patients, instability in the control of the blood sugar level appeared to us to merit primary consideration inasmuch as hypoglycemia and hyperglycemia, states between which the diabetic patient oscillates continuously, are both capable of stimulating the adrenal cortex (6–16). The present study was designed to determine whether there was any relation between the degree of control of blood sugar and the level of adrenal cortical activity. Serial measurements of the level of the blood sugar, the eosinophil count, the rate of urinary excretion of corticosteroids, and of certain other variables, have been made on a diabetic patient over 15-day periods during which crystalline insulin in dosage levels ranging from 25 to 80 units per day was administered.

EXPERIMENTAL PROCEDURE

The subject of these studies was a physically vigorous 19-year-old male in whom mongolism had been recognized for 18 years and diabetes mellitus for 12 years. For at least three years prior to these studies asymptomatic pyelonephritis had been present. White cells were continuously present in the urine, from which *B. pyocyaneus* could be cultured, despite repeated courses of sulfadiazine, streptomycin and aureomycin. However, no obstruction could be demonstrated in the urinary tract and the urea clearance test was normal. The renal threshold for glucose was elevated. Adrenal responsiveness was normal, as judged by the ACTH-eosinophil test.

At the time that these observations were made, the patient was 57.75 inches (144.4 cm.) tall and weighed 110 pounds (50 Kg.). Except for the short stature and mental retardation, positive physical findings consisted only of mongoloid features and hypertrophied tonsils. While he was at home, the patient's diabetes was reasonably well controlled on a 2,000 calorie diet by the administration of 40 units of protamine zinc insulin and 16 units of regular insulin in separate sites before breakfast. Insulin reactions had been rare and although he had become mildly acidotic on a few occasions, he had never been in diabetic coma.

These studies were conducted on a metabolic ward at intervals over a period of 12 months. The diet, which was maintained constant throughout, consisted of carbohydrate 193, protein 106, and fat 95 grams and provided 2,056 calories. Five grams of sodium chloride were added to the diet, making the total intake of sodium 126.1 mEq. per day, chloride 129.1 mEq., potassium 118.5 mEq., and nitrogen 16.9 grams per day. Four isocaloric feedings, together with an injection of crystalline insulin, were given at six-hour intervals. No limitation was placed upon the fluid intake. During periods when attempts were made to maintain the blood sugar within normal limits and insulin reactions occurred, 10 gram packets of glucose were provided for administration at the first indication of hypoglycemia. It was possible to

[1] This work was supported by grants from the National Institutes of Health, Public Health Service; the Committee on Research in Endocrinology, National Research Council; the Commonwealth Fund of New York and the American Cancer Society.

maintain the patient's physical activity relatively constant except during the spring season, when he frequently escaped observation and exercised vigorously out of doors. He was found several times in a semi-conscious state and on one occasion the use of intravenous glucose was required to terminate clonic convulsions. On this account the period of normoglycemia attempted during the spring was repeated during the winter when the amount of exercise taken could be more easily controlled. During the winter period constant surveillance of the patient was maintained by an attendant employed solely for this purpose, and light exercise only was permitted. Hypoglycemic reactions, which were few in number, were arrested promptly at their inception.

To facilitate insulin regulation the urine was collected in six-hour pools and a rapid quantitative estimation of the glucose content was made by the Somogyi (17) method. The dosage of crystalline insulin was so adjusted as to achieve as stable a degree of glycosuria (or, during aglycosuric periods, as stable a level of blood sugar) as possible during the selected regime of control. Five insulin dosage levels ranging from 25 to 80 units were employed over periods of 15 days each. Dosage levels were selected which would permit (a) maximal glycosuria without ketonuria, (b) moderate glycosuria, (c) aglycosuria with hyperglycemia, and (d) normoglycemia. (The normoglycemic regime was undertaken on two separate occasions for reasons previously explained.) Intervals of at least three weeks were permitted to elapse between the 15-day metabolic periods.

Quantitative analyses of the 24-hour urine specimens were made for creatinine and sugar. Specimens whose creatinine content indicated them to be complete [2] were either analyzed individually or, during certain periods, were combined into three-day pools for determination of the rate of excretion of sodium, chloride, potassium, nitrogen and corticosteroids. The blood sugar having been found to be minimal one hour before and maximal one hour after meals, the range of blood sugar during a given regime was established by frequent measurements taken at these times. An eosinophil count was made from venous blood drawn at 3 p.m. daily.

[2] While difficulty in securing complete collections was anticipated due to the patient's mental impairment, few specimens had, in fact, to be discarded.

METHODS

Sodium was determined by the method of Butler and Tuthill (18), chloride by a modification of the method of Wilson and Ball (19), potassium by the method of Fiske and Litarczek (20), nitrogen by the macro-Kjeldahl method of Peters and Van Slyke (21), and creatinine by a modification of the method of Bonsnes and Taussky (22). Blood and urinary glucose were estimated by the methods of Folin (23, 24). The urinary corticosteroids were measured by the method of Talbot, Saltzman, Wixon, and Wolfe (25).

Circulating blood eosinophils were counted in the double-celled Fuchs-Rosenthal chamber by a modification of the method of Randolph (26). Two U. S. Bureau of Standards certified pipettes were employed, and all of the eosinophils in both cells of four chambers counted.

RESULTS

The effect which the different dosage levels of insulin exerted upon the blood glucose, urinary glucose excretion, urine volume, eosinophil count and urinary corticosteroid excretion is summarized in Table I; the effect upon the body weight and the urinary excretion of nitrogen and electrolytes is summarized in Table II.

During the period of maximal glycosuria the urine volume increased over that observed during

TABLE I

The effect of various degrees of control upon the blood and urine glucose, the urine volume, the eosinophil count and the corticosteroid excretion rate of a diabetic patient

Regime	Mean dosage of crystalline insulin (*U/24 hrs.*)	Range of blood glucose (*mgm. %*)	Mean urinary glucose excretion (*gm./24 hrs.*)	Mean urine volume (*cc./24 hrs.*)	Eosinophil count at 3 p..m (*cells/mm.³*)		Mean corticosteroid excretion rate (*mgm./24 hrs.*)
					Mean	S.D.	
Maximal glycosuria without ketonuria	25	328–461*	124.3	3,212	190	25.5	0.20
Moderate glycosuria	39	270–445†	58.2	2,721	182	18.8	0.26
Aglycosuria with hyperglycemia	55	244–407†	0	2,780	197	17.5	0.25
Normoglycemia (spring)							
(a) Days 1–5	70	43–214†	0	3,230	—	—	2.81
(b) Days 6–10	79		0	3,786	—	—	4.07
(c) Days 11–15	77		0	4,528	—	—	5.64
Normoglycemia (winter)							
(a) Days 1–5	85	33–155†	0	3,553	—	—	0.18
(b) Days 6–10	81		0	3,635	—	—	0.34
(c) Days 11–15	74		0	4,014	—	—	0.54

* Venous. † Capillary.

TABLE II

The effect of various degrees of control upon the urinary excretion of electrolytes and nitrogen by a diabetic patient

Regime	Mean dosage of crystalline insulin (*U/24 hrs.*)	Sodium (*mEq./24 hrs.*)		Chloride (*mEq./24 hrs.*)		Potassium (*mEq./24 hrs.*)		Nitrogen (*gm./24 hrs.*)		Change in Body weight (*Kg.*)
		Mean	S. D.	Mean	S. D.	Mean	S. D.	Mean	S. D.	
Maximal glycosuria without ketonuria										
(a) Days 1–5	25	135.0	36.7	131.5	29.3	91.3	13.5	17.1	1.6	−1.20
(b) Days 6–10	25	116.8	12.0	122.6	17.1	89.7	3.9	16.8	0.6	−0.32
(c) Days 11–15	25	107.8	7.2	113.5	3.0	88.7	6.6	17.2	1.3	−0.30
Moderate glycosuria	39	114.1	6.9	116.4	5.6	86.0	2.9	15.1	0.3	+0.22
Aglycosuria with hyperglycemia	55	104.7	11.2	117.2	11.0	86.3	6.9	14.4	0.7	+0.67
Normoglycemia (spring)	75	99.5	4.5	110.3	7.9	75.8	2.4	13.5	0.8	+0.32
Normoglycemia (winter)	80	104.5	7.0	109.0	5.3	75.8	7.4	13.0	1.2	+0.78

periods of moderate glycosuria and aglycosuria with hyperglycemia. Wide fluctuations in daily glucose excretion occurred despite the precautions taken to achieve stability. In Table II, the data for the period of maximal glycosuria have been broken down into three five-day subdivisions in order that the progressive changes which took place in the rate of excretion of sodium and chloride may be apparent. The sudden initiation of glycosuria by a reduction in insulin dosage from 42 to 29 units on the day before the period began was accompanied by an appreciable loss of urinary sodium and chloride. The rate of salt excretion declined during the middle third, and became normal during the final third of the period. The serum sodium and chloride, which remained essentially stationary during all other regimes, fell during the regime of maximal glycosuria. Over the 15 days, the serum sodium declined from 141.6 to 135.5 and the chloride from 100.7 to 97.0 mEq. per liter. Excretion rates for potassium and nitrogen rose to the highest levels observed during the study and remained relatively constant throughout the three subdivisions of the period. The nitrogen balance was consistently negative, whether fecal nitrogen loss was estimated at 10% of the nitrogen intake or at 1.283 grams per 24 hours, as suggested by Reifenstein, Albright, and Wells (*27*). Body weight declined rapidly during the first third of the period and more slowly during the remainder. Since no significant changes in the rate of nitrogen excretion occurred, the precipitous early fall in body weight was presumably associated with a loss of extracellular fluid. Despite the loss of salt and nitrogen and the polyuria, the eosinophil count and urinary corticosteroid excretion rate remained within normal limits.

The period of moderate glycosuria was associated with a reduction in the urine volume to the rather large value which was usual for the patient. The rate of sodium and chloride excretion did not differ appreciably from that observed during the final third of the period of maximal glycosuria or during subsequent periods of more rigorous control. The rate of nitrogen and potassium excretion decreased, the nitrogen balance became positive, and a small gain in weight occurred. No significant alteration was observed in the eosinophil count or urinary corticosteroid excretion rate.

During the period of aglycosuria with hyperglycemia, the blood sugar occasionally rose to moderately high levels without a loss of sugar in the urine. The rate of nitrogen excretion decreased still further and weight was gained more rapidly. The eosinophil count and corticosteroid excretion rate remained essentially unchanged.

The two periods of attempted normoglycemia were accompanied by the excretion of large volumes of urine, in amounts which exceeded even those observed during the period of maximal glycosuria. The rate of excretion of nitrogen and potassium declined to the lowest levels observed during these studies. Despite frequent estimations of the level of blood sugar and adjustments of insulin dosage, hypoglycemic reactions often occurred, particularly during the spring when the patient escaped observation. Eosinophil counts became so erratic that representative values could no longer be obtained serially, the eosinophils falling after insulin reactions and subsequently rebounding to high levels. The rate of cortico-

steroid excretion increased progressively during the normoglycemic regimes, attaining values of 5.64 mgm. and 0.54 mgm. per 24 hours at the conclusion of the spring and winter regimes, respectively.

DISCUSSION

(*a*) *Measurements of adrenal cortical activity.* The observation that adrenal cortical activity, as judged by the eosinophil count and the rate of corticosteroid excretion,[3] did not vary in the three regimes characterized by consistent hyperglycemia is in accord with previous studies. Thus, when the present patient was subjected to total insulin deprivation, no indication of adrenal hyperactivity could be demonstrated until mild ketosis and acidosis became manifest (28). Similar findings have been obtained after the withdrawal of insulin from depancreatized dogs (29). Although the administration of glucose has occasioned eosinopenia in acute experiments (16), the induction of a more gradual rise in the blood sugar apparently need not lead to an increase in the rate of corticosteroid excretion. The only finding which suggests the occurrence of adrenal hyperactivity during the hyperglycemic regimes is the striking conservation of urinary sodium and chloride which was gradually effected during the period of maximal glycosuria.[4] While this economy may have been achieved through the activation of an idiorenal mechanism, it is possible that increased secretion of an adrenocortical "salt" hormone was responsible.

The finding that adrenal hyperactivity accompanied the normoglycemic regimes, with their recurring insulin reactions, agrees with many previous observations concerning the physiological effects of hypoglycemia. However, the magnitude of the rise in corticosteroid excretion which occurred during the spring regime occasioned some surprise.

The moderate increase in the amount of physical activity taken by the patient during the spring regime undoubtedly influenced adrenal secretory activity somewhat. However, it seems unlikely that this factor alone could account for the heightened rate of corticosteroid excretion during the spring, as compared with the winter, regime. The amount of exercise taken was far less than that habitually enjoyed by mentally normal boys of the same age. The fact that no increase in insulin dosage was required to preserve approximate normoglycemia was unanticipated; it had been expected that adrenal hyperactivity of the degree observed would lead to insulin resistance.

(*b*) *General metabolic observations.* The loss of sodium and chloride which occurred during the early phases of the period of heavy glycosuria was anticipated from the results of previous acute experiments (31–33). The conservation of salt which was gradually effected despite continuing glycosuria was, however, unexpected. The mechanism by which such conservation is accomplished merits investigation.

The large urine volume observed during the periods of attempted normoglycemia was conceivably a manifestation of "stress." Both Lloyd (34) and Corcoran, Dustan, and Page (35) have noted the existence of a positive coefficient of correlation between urine volume and corticosteroid excretion rate.

The inverse relationship found to exist between the rate of urinary nitrogen and potassium excretion and the dose of insulin administered presumably reflects the capacity of insulin to accelerate the rate of protein synthesis (36–42).

In so far as it is permissible to generalize from studies on a single patient, these short-term descriptive experiments suggest that the rigor of the therapeutic regimen may exert a significant effect upon adrenal cortical activity in the diabetic patient. Strict regimes of control, with their tendency to recurrent insulin reactions, appear to be accompanied by an accelerated rate of release of corticosteroids. Lenient regimes appear to be associated with increased corticosteroid secretion only when prolonged hyperglycemia has led secondarily to ketoacidosis. That regimes permitting

[3] Until the intermediary metabolism of steroids has been more completely clarified, the possibility that corticosteroid excretion may be low despite high secretion because of rapid tissue utilization must, of course, be conceded.

[4] Had ketonuria been permitted progressive base conservation could not, in all probability, have been demonstrated (note the continuous loss of base by Atchley's and associates' [30] patient W. O'C. during 11 days' total insulin deprivation).

heavy glycosuria may be accompanied by an accelerated rate of release of mineralocorticoids is suggested, although not proved, by the data presented.

The long-term effects of therapeutic regimes of different severity can only be surmised. It is conceivable that maintenance of a high level of adrenal cortical function reduces the quantum of "adaptation energy" (43) available to meet extraneous stresses and predisposes ultimately to adrenal exhaustion. In this connection, the observation of Field and Marble (5) that there is an inverse correlation between the duration of diabetes and the degree of adrenal cortical responsiveness to surgical trauma may be pertinent.

The maintenance of a high concentration of adrenal cortical hormones in the circulation of a diabetic patient almost certainly exerts widespread biologic effects; whether the over-all consequences of these effects are beneficial or harmful to the diabetic organism remains to be determined by painstaking investigation.

SUMMARY

Crystalline insulin in dosage levels ranging from 25 to 80 units was administered for periods of 15 days to a patient with mongolism and diabetes mellitus while he was maintained on a constant diet and unlimited fluids. Dosage levels were selected which would permit, (a) maximal glycosuria without ketonuria, (b) moderate glycosuria, (c) aglycosuria with hyperglycemia, and (d) normoglycemia. The level of adrenal cortical function was estimated by means of serial eosinophil counts and urinary corticosteroid determinations, and the rates of urinary excretion of nitrogen, sodium, chloride, and potassium were also measured.

The dosage of insulin was found to exert a significant influence upon the rate of corticosteroid excretion and the eosinophil count, as well as upon the rates of excretion of water, electrolytes, and nitrogen. The administration of large doses of insulin in attempts to insure continuous normoglycemia resulted in the occurrence of frequent insulin reactions and in measurable increases in the rate of corticosteroid excretion. The administration of small doses which just prevented ketonuria exerted no detectable effect upon the rate of corticosteroid excretion or the eosinophil count although a heavy glycosuria ensued. That an adrenocortical "salt" hormone was perhaps secreted during this regime is suggested by the fact that conservation of sodium and chloride was gradually effected despite continuing glycosuria.

Urine volumes were greatest during the periods of attempted normoglycemia when they exceeded even those observed during the period of maximal glycosuria. There were no significant alterations in sodium and chloride excretion rates except during the initial period of maximal glycosuria. During the early phases of this period a loss of salt accompanied that of glucose. The rate of excretion of potassium and nitrogen varied inversely with the dose of insulin administered.

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FACTORS INFLUENCING CHOLINE ABSORPTION IN THE INTESTINAL TRACT[1]

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Previous investigations (1) have demonstrated that in normal persons approximately 60 per cent of choline salts fed in a single dose is transformed by intestinal bacteria to trimethylamine (TMA), which is excreted in the urine mainly as the oxide. In chronic liver diseases, considerably less of the ingested choline appears in the urine as total trimethylamines (TTMA). This difference was considered as evidence of an altered action of the intestinal bacteria in these diseases (1). The concept of the intestinal formation of urinary trimethylamines, based in part upon the reduction of TTMA excretion following administration of antimicrobial agents, was confirmed by the observation (2) that choline as such appears in the urine after intravenous administration of choline, but the basal excretion of urinary TTMA is not altered significantly. The concept of altered choline utilization by intestinal bacteria in liver diseases, as reflected by reduced transformation of choline to TMA, required further elaboration. This was partly carried out in animal experiments (3), but in addition it was necessary to perform a series of clinical studies to clarify the results of human and animal investigations reported previously. Studies of the physiologic processes included the following: (a) whether choline bound in the form of phospholipids is transformed to TMA to the same degree as are choline salts, (b) investigation into the site and completeness of intestinal transformation of choline, and (c) determination of the effects of aureomycin upon the transformation of large amounts of choline. Studies of the alteration of intestinal transformation of choline in liver disease were concerned with (a) the specificity of reduced TTMA excretion in hepatic disease, (b) the effect of large amounts of choline upon the TTMA excretion in cirrhosis, and (c) the experimental alteration of intestinal flora by prolonged feeding of choline on the premise that bacteria, such as *Proteus vulgaris* known to transform choline (4, 5), would increase at the expense of such bacteria as *Escherichia coli* and coliform paracolons which do not utilize this substance.

[1] Supported by a grant from the Doctor Jerome D. Solomon Memorial Research Foundation.

MATERIAL AND METHODS

Patients were selected with hepatic diseases, particularly cirrhosis, and non-hepatic diseases such as pneumonia, leukemia, cancer, and rheumatoid arthritis. Normal persons (staff of the Hektoen Institute) served as controls. Urine and stool samples were collected for TTMA or choline determination after oral administration of a test dose of choline as bicarbonate.[2] In all urine samples, the TTMA were determined by treating the urine with Devarda's alloy followed by aeration (1). The total 24-hour excretions were expressed as TTMA N. Not taken into account was the basal urinary TTMA excretion, which varies from 5.7 to 15.0 mg. N in 24 hours (1), but is relatively constant with the same diet. Four normal persons and two patients with cirrhosis were given 20 grams of a purified lecithin orally which was found to contain 230 mg. choline nitrogen, determined according to the technic of Handler (6). Urine specimens were collected 24 hours prior to and for two consecutive 24-hour periods immediately following administration of lecithin. In three cirrhotics, the contents of different levels of the intestinal tract were obtained by a Miller-Abbott tube. The ability of these contents to transform choline to TMA was tested, as described previously, by incubation of a small amount in a tryptose medium containing 1 per cent choline chloride (1). Total bacteria counts were also done on these contents. In an additional experiment 10 ml. of the same medium were inoculated with stools of two normal persons and three patients with cirrhosis, and choline and trimethylamine concentrations were determined and compared after incubation. In two instances, 4 grams of choline base as bicarbonate were given orally, together with 0.5 gram of carmine as a stool marker, and total feces were collected. Fractional stools were kept in a frozen state until the total feces were obtained. The feces were homogenized, weighed, and 100 grams extracted with 100 ml. of alcohol. After cen-

[2] Thanks are due to Commercial Solvents Corporation for a generous supply of the choline salts used in these experiments.

trifugation the solids were re-extracted four times in the same way. The total alcohol extracts were evaporated and the residue taken up in 25 ml. N hydrochloric acid. This was treated with Norit A and allowed to stand for one hour. After filtration an aliquot was taken for determination of choline, as described previously for urine (1).

In two patients with non-hepatic disease, the urinary TTMA were determined after a test dose of 10 grams of choline base. The patients were then given 0.5 gram of aureomycin orally six times daily for five consecutive days. The test dose of choline was repeated during the last day of aureomycin administration and the urinary TTMA determined. In another series three normal persons received 4 grams of choline base twice one week apart, the second time with the addition of 500 mg. aureomycin. Urinary TTMA excretion was determined on the first and second day following choline administration.

Nine normal persons, 39 patients with hepatobiliary disease, especially cirrhosis, and 11 patients suffering from different severe non-hepatic disease received 2 grams of choline base (230 mg. choline N). The urines subsequently collected for a 24-hour period were examined for TTMA.

In two patients with cirrhosis, the TTMA excretion following the intake of 2 grams of choline base was determined five days prior to and after the administration of 5 grams of choline base twice a day for two consecutive days. An interval of five days was chosen to allow the return to basal TTMA excretion before administration of the second test dose.

Nine patients with cirrhosis were treated for two weeks with three daily oral doses of 2 grams of choline base as choline citrate, and the TTMA excretion was redetermined five days after discontinuance of treatment. Total bacteria counts, as well as the percentage of *E. coli* and coliform paracolons, were determined before and following choline treatment. The stools were weighed and thoroughly homogenized with saline under sterile precautions. Serial dilutions from 10^{-5} to 10^{-15} were prepared. Duplicate plates with tryptose agar and eosin-methylene blue medium (Difco) were inoculated with each dilution, using the pour-plate technic for the first, and the surface-swabbing method for the second type of plate. Twenty-four and 48 hours later the number of bacterial colonies was counted and representative members of each colony type identified by morphologic, tinctorial and biochemical properties, according to the classification of Bergey's manual (7). In one patient in this group the bacteriologic studies were repeated and in three other patients the determination of urinary TTMA after a test dose of 2 grams of choline was repeated two to three weeks after the discontinuance of prolonged choline administration. In all nine patients in this group, the cephalin flocculation, thymol turbidity, serum albumin, cholesterol ester, total cholesterol ratio, and lipid phosphorus (as determined by standard technics) were compared prior to and following choline administration.

RESULTS

1. Study of physiologic processes

a. Lecithin administration. Urinary excretion of TTMA following the intake of an amount of lecithin containing 2 grams of choline base was considerably lower than following the intake of the same amount of choline in the form of bicar-

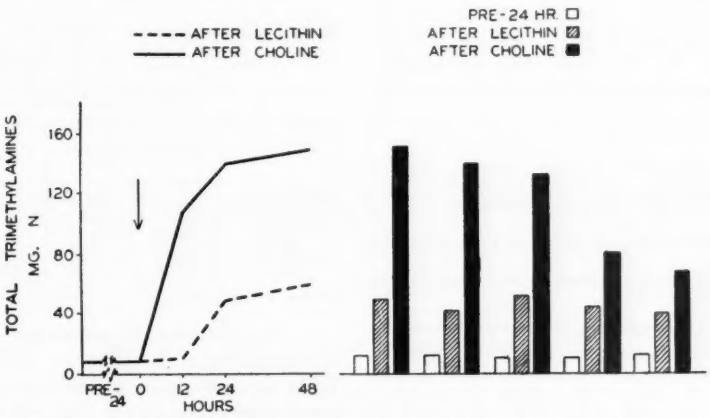

FIG. 1. EXCRETION OF TOTAL TRIMETHYLAMINES BEFORE AND IN THE 24-HOUR PERIODS FOLLOWING ORAL INTAKE OF CHOLINE AND LECITHIN CONTAINING THE SAME AMOUNT OF CHOLINE NITROGEN (230 MG. N)

The curve and the first three groups of bars represent four normals, the last two bars two patients with hepatic cirrhosis.

TABLE I

Total bacterial counts and per cent of choline in medium transformed to trimethylamine by the contents of different levels of the gastro-intestinal tract in three patients with hepatic cirrhosis

Site or distance (*in feet*) from pylorus	Case 1		Case 2		Case 3	
	Bacterial count (*per ml.*)	Per cent choline transformed	Bacterial count (*per ml.*)	Per cent choline transformed	Bacterial count (*per ml.*)	Per cent choline transformed
Stomach	881	38	11,520	50	1,400	40
Duodenum	2	0	0	0	—	—
1	4,280	25	5,760	42	120	35
2	1,037	47	1,280	37	500	46
3	1,719	43	10,386	45	—	—
4	437	45	6,912	42	700	58
5	4,398	49	1,024	51	—	—
6	5,372	54	1,792	44	70	30
7	5,732	59	382	47	—	—

bonate (Figure 1). In addition, the bulk of TTMA appeared in the urine in 12 hours or less after ingestion of choline as such, whereas after ingestion of lecithin, the bulk of TTMA appeared in the urine between 12 and 24 hours. In cirrhosis, ingestion of lecithin is followed by the same TTMA excretion as in normals. This is in contrast to the decreased TTMA excretion in cirrhosis after choline intake. If the basal TTMA excretion is deducted, the 24-hour TTMA excretion in normal persons after lecithin ingestion was only 26.1 per cent of the TTMA excretion after ingestion of equivalent amounts of choline; in the two cirrhotics it was 50.1 per cent of the excretion after choline.

b. Transformation of choline by gastro-intestinal content. In the three instances studied, choline was transformed to TMA by the contents of the stomach and small intestine, except in the duodenum. This ability to transform choline paralleled the presence of bacteria (Table I). Choline-containing culture media inoculated with the stool of two normals and three cirrhotics revealed, after incubation, a sum of choline and TMA N equivalent to the choline N originally present (Table II). The TMA concentration of a TMA-containing medium did not change after inoculation with feces, indicating that intestinal bacteria transform choline only to TMA and not to a substance unaccounted for by the method used. In neither one of the normal persons who had taken orally 4 grams of choline with 100 grams of starch (which has been shown [1] to suppress intestinal TMA formation) nor in the one cirrhotic who excreted low amounts of TTMA after choline could significant amounts of choline (not more than 4 per cent of the dose given) be demonstrated in the feces.

TABLE II

Choline and trimethylamine nitrogen content of a tryptose medium (containing 1 mg. choline nitrogen per ml.) inoculated with stools and incubated for 24 hours at 37°C

The control represents an incubated but not inoculated medium.

Diagnosis	Medium		
	Choline N	TMA N	Choline + TMA N
	mg./ml.	*mg./ml.*	*mg./ml.*
Normal	0.47	0.55	1.02
Normal	0.50	0.50	1.00
Cirrhosis	0.45	0.57	1.02
Cirrhosis	0.37	0.61	0.98
Cirrhosis	0.53	0.49	1.02
Control	0.00	0.98	0.98

TABLE III

Influence of aureomycin treatment upon urinary excretion of total trimethylamines after oral administration of 10 grams choline base in the form of bicarbonate

Diagnosis	TTMA excretion (*mg. N per 24 hours*)	
	Before aureomycin treatment	During aureomycin treatment
Tuberculosis of hip	802.0	6.3
Fracture	670.0	116.6

TABLE IV

Urinary excretion of total trimethylamines of three normals after intake of 4 grams choline base (equivalent to 460 mg. choline N) with and without simultaneous oral administration 500 mg. aureomycin

Hours after choline intake . .	Without aureomycin (*mg. TTMA N*)		With aureomycin (*mg. TTMA N*)	
	0–24	24–48	0–24	24–48
No. 1	230.0	34.5	47.0	10.8
No. 2	240.0	36.0	71.0	25.8
No. 3	222.0	31.0	87.0	—

c. Administration of aureomycin. Even after administration of 10 grams of choline base, the TTMA excretion was decidedly reduced if aureomycin were given (Table III). In the two cases studied, the amount excreted was lower than the usual excretion of TTMA after administration of 2 grams of choline in the absence of aureomycin, and in one it was below normal basal excretion of

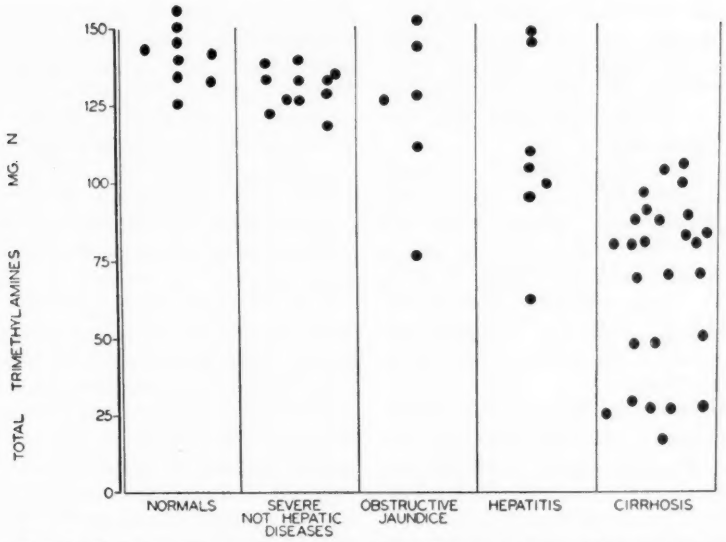

FIG. 2. DISTRIBUTION OF THE AMOUNT OF TOTAL TRIMETHYLAMINES EXCRETED IN THE URINES IN THE 24-HOUR PERIOD FOLLOWING THE INTAKE OF 2 GRAMS OF CHOLINE BASE (230 MG. N) IN NORMALS AND IN PATIENTS WITH VARIOUS DISEASES

about 10 mg. TTMA nitrogen in 24 hours. The administration of a single small dose of aureomycin together with choline suppressed the TTMA excretion markedly, even on the second day after administration (Table IV).

2. Alteration of intestinal transformation of choline in diseases

a. Specificity of reduced TTMA excretion for liver disease. The histogram (Figure 2) shows clearly that in cirrhosis, TTMA excretion after a test dose of choline was significantly and consistently reduced in comparison with normals, whereas in acute hepatitis and particularly in obstructive jaundice the reduction was less constant. In patients with severe disease without apparent hepatic involvement, the excretion was almost within the normal range.

b. Effect of large doses of choline. The TTMA excretion after a test dose of choline in two cir-

TABLE V

Influence of prolonged treatment with choline upon urinary total trimethylamines excretion in patients with cirrhosis of liver after oral administration of choline bicarbonate corresponding to 230 mg. N, upon the total stool bacterial count, and upon the percentage of colon bacilli and coliform paracolons from the total stool bacteria

TTMA excretion (*mg. N per 24 hours*)			Total bacterial count (*per gm. stool*)			Colon bacilli and coliform paracolons (*percentage of total count*)		
Before choline treatment	5 days after	20 days after	Before choline treatment	5 days after	20 days after	Before choline treatment	5 days after	20 days after
87.9	156.8		5.1×10^{8}	7.9×10^{7}		14.7	5.7	
113.0	148.0		8.6×10^{8}	8.0×10^{7}		17.8	16.3	
82.1	148.5		3.5×10^{11}	1.3×10^{11}		65.2	43.2	
49.5	141.9		2.4×10^{11}	7.9×10^{12}		54.1	0.7	
48.5	126.6		1.1×10^{10}	2.7×10^{11}		7.3	0.1	
96.5	141.7		5.2×10^{9}	4.3×10^{8}		27.8	0.4	
73.0	146.0	117.0	2.1×10^{8}	2.4×10^{8}	2.9×10^{8}	10.5	6.2	62.5
91.2	157.7	82.0						
88.0	129.5	85.0	1.8×10^{8}	2.5×10^{8}		30.4	11.3	

rhotics, who had previously received 20 grams of choline in two days, did not vary significantly from their excretion following a test dose (69.5 and 27.2 mg. TTMA N without previous choline treatment in 24 hours compared with 62.0 and 33.0 mg. afterwards).

c. Influence of prolonged choline treatment. Following prolonged daily administration of choline to nine patients with cirrhosis (with and without jaundice), the TTMA excretion after a test dose of choline, which was initially reduced, rose to normal. These differences were significant in all instances (Table V). No significant differences occurred in the results of the hepatic tests during this period.

In three cases in whom a third test dose of choline was given from two to three weeks after discontinuance of the daily choline treatment, the urinary TTMA fell again to almost the level before treatment. The total bacterial count did not change significantly in eight cases in which bacterial studies of the stool were performed. During treatment, the percentage of the coliform bacteria dropped markedly in four instances, moderately in three, and only slightly in one. In the one instance in which the investigation was repeated several weeks after discontinuance of daily choline treatment, the percentage of coliform bacteria had risen significantly.

DISCUSSION

The experiments presented were designed to clarify the phenomena described previously (1), that intestinal bacteria destroy the bulk of intestinal choline and that this process is reduced in liver disease. It has been shown that intestinal bacteria do not degrade choline to nitrogenous substances other than trimethylamine. Ingested trimethylamine chloride is almost quantitatively excreted in the urine (1), indicating that it undergoes no degradation in the body once it is formed from choline. No matter how low the urinary TTMA excretion is, the feces do not contain significant amounts of choline in humans or rats (3). This indicates that low TTMA excretion is not due to loss of choline in the stool. These observations lead to the conclusion that reduced urinary TTMA excretion in liver disease is associated with increased choline absorption. That this is a specific occurrence in chronic liver disease is supported by the observations that in severe systemic disease without significant liver involvement, TTMA excretion is normal, and that in animal experiments significant reduction of urinary TTMA excretion occurs only in chronic but not in acute hepatic injury (3). The reduced urinary TTMA excretion in chronic liver disease can be explained either by increased absorption of choline (preventing a larger portion than normal from being destroyed by intestinal bacteria) or by a primary alteration of the bacteria so that they utilize less choline and more remains to be absorbed. The important role of bacteria is suggested by the significant reduction of the TTMA excretion by simultaneous administration even of single doses of aureomycin with choline. Topographically, bacterial degradation of choline starts very high in the intestine, near a level where choline absorption can be assumed to begin from our knowledge of lipid absorption (8). At first thought, the hypothesis of primary alteration of bacterial activity is also supported by observations on the effect of prolonged choline treatment. This treatment did not alter hepatic function, but elevated the abnormally low TTMA excretion to the normal level, and discontinuance of the therapy resulted in a return to subnormal levels. If bacterial determinations in the stools can be relied upon to reflect changes of bacteria in the intestine, elevation of TTMA excretion after prolonged choline treatment in these studies was associated with a significant decrease of the percentage of non-choline-utilizing bacteria. However, there is no evidence that the intestinal flora contain an abnormally high percentage of non-choline-utilizing bacteria in liver disease. It is possible, therefore, that these experiments do not represent a correction of an abnormal process, but an unusual overload of the intestinal content favoring more rapid multiplication of the bacteria which transform choline. A primary alteration of intestinal choline absorption would be supported by the finding that when choline is given as lecithin, much less is excreted as TTMA than when the same amount is given as free choline. It is possible that choline absorption is associated with lecithin formation in the intestinal wall. Choline deficiency in the patients, possibly associated with an increased

avidity for choline, could be excluded as the cause of altered TTMA excretion in liver diseases, since temporary overloading of the body with choline by administration of large amounts for a short time did not influence the reduced TTMA excretion.

The evidence presented here, therefore, strongly suggests an increased absorption of choline in chronic liver disease, but whether this is the result of altered bacterial action or altered permeability of the intestinal wall remains to be established.

SUMMARY

Attempts have been made to elaborate upon the observation reported previously that the bulk of choline administered orally is transformed in the intestinal tract to trimethylamine, which is excreted in the urine mainly as the oxide, and that this excretion is diminished in liver disease (1). The transformation by intestinal bacteria starts in the upper part of the intestinal tract. Choline not so transformed is assumed to be absorbed, since intestinal bacteria are unable to transform choline to nitrogenous substances other than trimethylamine and no significant amounts of choline could be found in the stool, even when trimethylamine formation was depressed.

Trimethylamine formation can be changed by alteration of bacterial flora. Choline, in amounts as large as 10 grams, can be protected from bacterial action by the administration of aureomycin; the administration of a single dose of 0.5 gram of aureomycin significantly reduced the transformation of 4 grams of choline base. Oral administra- of choline for two weeks usually results in depression of the percentage of non-choline-utilizing bacteria in the stool and leads regularly to an increase of the originally reduced trimethylamine excretion in liver disease. Discontinuance of treatment results in a decrease of trimethylamine excretion to initial levels.

The reduction of trimethylamine excretion appears specific for liver disease; it was not found in other severe diseases studied.

The TTMA excretion does not increase as much after the administration of lecithin as after the administration of an equivalent amount of choline, suggesting that a greater amount of choline is absorbed if it is given in bound form. It is tentatively assumed that choline absorption is increased in liver disease.

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THE POSSIBLE USE OF NEUTRON-CAPTURING ISOTOPES SUCH AS BORON^{10} IN THE TREATMENT OF NEOPLASMS. II. COMPUTATION OF THE RADIATION ENERGIES AND ESTIMATES OF EFFECTS IN NORMAL AND NEOPLASTIC BRAIN [1]

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The nuclei of the principal atoms found in normal living tissue have but little tendency to capture very slow (thermal) neutrons, whereas the nuclei of a certain few isotopes have a remarkable propensity for capture of thermal neutrons. Among the isotopes in the latter group, boron^{10} (B^{10}) attracted the attention of Kruger (1) and of Zahl, Cooper, and Dunning (2) as early as 1940 because the capture reaction yields a high energy alpha particle which dissipates all its energy in tissue within *circa* 14 μ. The reaction may be written:

$$B^{10} + n \rightarrow [B^{11}] \rightarrow Li^{7} + \alpha^{++} \text{ (2.4 mev.)}.$$

These writers pointed out that if B^{10} were concentrated in a neoplasm which was then irradiated with slow neutrons, the tumor might be destroyed. Since the destructive effect of the α particle will be limited either to the cell containing the parent atom of B^{10} or to cells in its immediate neighborhood, normal cells are likely to survive.

If this idea is to bear fruit in treatment of tumors in man, we need: *1*) a powerful source of slow neutrons, *2*) a method for achieving a high differential concentration of boron in tumor as compared with normal tissue, and *3*) information as to the absolute concentration of the boron in various tissues which would be essential for destruction of only the neoplastic cells by the energies consequent upon the neutron beam. Of these requirements No. *1* is now supplied by the intense beam of slow neutrons emerging from the nuclear reactor at the Brookhaven National Laboratories. Sweet and Javid (3) have recently described steps toward meeting the second requirement insofar as intracranial tumors are concerned. Briefly, it was found that if borax is injected intravenously it will appear in many rapidly growing brain tumors in ratios of $> 3:1$ as compared with normal brain. A maximal differential of 48:1 was recorded in one case. The peak ratios are usually present only during the first 15–30 minutes after injection. Clinical analyses indicate that the actual concentration attained in the brain tumors after the nontoxic dose of 15 g. borax/70 Kg. body weight is > 50 μg. B/g. of tumor, whereas the normal gray matter contains only about 15 μg. B/g. of tissue. The normal white matter contains even less. The amounts found in blood are insufficient to account for the larger concentration in tumor. The scalp contains less than the blood, tumor, or muscle. Figure 1 shows the variations in concentrations in boron in six types of tissue taken in a series of biopsies during operative removal of a glioblastoma multiforme. (The colorimetric method of Ellis, Zook and Baudisch [4] was used for the boron determinations.[3])

We also present here our moves toward requirement No. *3*, an estimate of the energies released and of their effects, but before we do so a summary of the pertinent background facts of nuclear physics in relation to biology may be helpful.

Most of the nuclear radiations, *i.e.*, gamma rays, beta particles, alpha particles (helium atoms with a double positive charge), protons and fast neutrons, are usually produced at radioactive decay with large energies ranging up into a few millions of electron volts (mev.). When rays

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[2] Dr. Javid was a Research Fellow in Surgery, Harvard Medical School when this work was done.

[3] We are indebted to Dr. Ellis for supplying us with the special dye required, 1:1′ dianthrimide, and for helpful advice.

or particles at these high energies react with matter dissipating their energies, the net result is the displacement of orbital electrons ionizing the remaining atom, with a potentially lethal effect on neighboring living tissue. These reactions are relatively nonspecific and most of the atoms of matter participate in them.

However, thermal neutrons, those whose speed has been cut down to about 2,200 meters/sec., leaving them with an energy of only .025 electron volts (ev.), interact with matter in an entirely different and much more specific fashion. Their energy, and that of the so-called epithermal neutrons moving somewhat faster, is insufficient to produce ionization, since approximately 10 electron volts is required to displace an orbital electron. Neutrons at these lower energies react with the atoms in living tissues largely by undergoing capture by the nuclei of certain of the atoms they meet.

An atomic nucleus that has captured a neutron becomes a new compound nucleus with an excess of energy which it immediately gives up in one of two major ways by:

(*1*) Emission of a capture radiation (gamma ray or heavy particle) forming a stable isotope.

$$\text{hydrogen}^1 + \text{neutron} \rightarrow [\text{hydrogen}^2] \rightarrow \text{hydrogen}^2 + \text{gamma ray}$$

$$H^1 + n \rightarrow [H^2] \rightarrow H^2 + \gamma \ (2.23 \text{ mev.})$$

$$\text{boron}^{10} + \text{neutron} \rightarrow [\text{boron}^{11}] \rightarrow \text{lithium}^7 + \text{alpha particle}$$

$$B^{10} + n \rightarrow [B^{11}] \rightarrow Li^7 + \alpha^{++} \ (2.4 \text{ mev.})$$

(*2*) Emission of a capture radiation (gamma ray or heavy particle—in the example below, a proton), forming a radioactive daughter which then continues to emit a further radiation at its characteristic rate.

$$\text{nitrogen}^{14} + n \rightarrow [\text{nitrogen}^{15}] \rightarrow \text{proton} + \text{carbon}^{14} \xrightarrow[5{,}700 \text{ years}]{\text{half life}} \text{nitrogen}^{14} + \text{electron}$$

$$N^{14} + n \rightarrow [N^{15}] \rightarrow p^+ + C^{14} \rightarrow N^{14} + \beta^-$$

Both the immediate "capture" radiations and the "decay" radiations from any radioactive daughter are ionizing and capable of producing a biologic effect which must be estimated for every type of atom within any tissue to be irradiated with slow neutrons. Although the neutrons arising in the nuclear reactor as the consequence of the fission of uranium are given off at high speeds, their velocity can be reduced by collisions within graphite or heavy water until the thermal neutron range is reached. Furthermore since the neutron carries no electrical charge it wanders more freely in matter than do the charged particles.

The potential usefulness of such radiations depends upon the remarkable eagerness of a few unusual isotopes to capture slow neutrons while all of the common naturally occurring elements in living tissue tend relatively to ignore them. The avidity of an isotope for absorption of slow neutrons is expressed as its capture cross section σ_a, the apparent area in cm.^{2} presented by a single nucleus of the element to an incident beam of neutrons, = "barns"

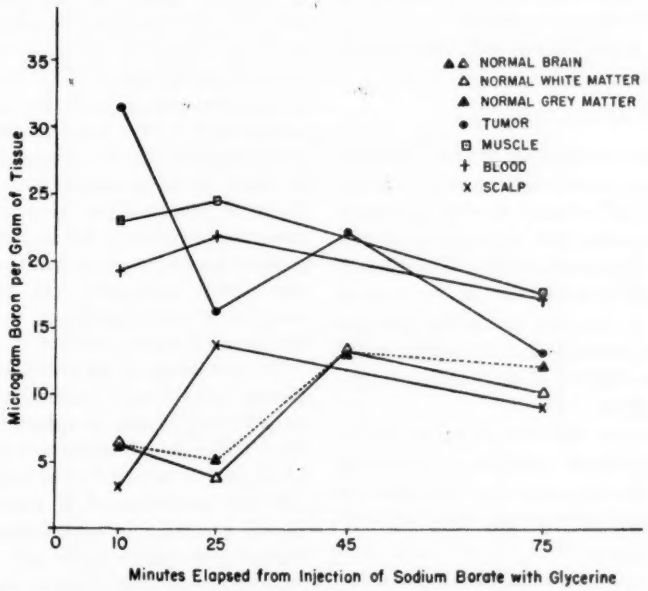

FIG. 1. VARIATIONS IN BORON CONCENTRATION IN TISSUES AFTER I.V. INJECTION IN PATIENT A. M.

Five g. sodium borate, containing 0.57 g. boron, injected.

$\times 10^{-24}$ cm^2. The σ_a for the main elements in normal tissue is as follows: H = .32, N = 1.7, O = <.001, P = .15, Cl = 32.5, Na = .45, C = .0045, Mg = .07, S = .5, K = 2.05, Ca = .42. In contrast with these low values are those for a limited number of isotopes among all the others to be found in matter. Thus Li^6 = 870; B^{10} = 3,900; Cd^{113} = 24,000; Sa^{149} = 46,000; Eu^{155} = 14,000; Gd^{157} = 200,000; Dy^{164} = 2,620; Hg^{199} = 2,500.

We now give our computation of the roentgen equivalent physical/min. (rep/min.) evolved in brain tumor and in normal gray and white matter containing the previously mentioned amounts of boron when such tissues are subjected to a specified flux of thermal neutrons. In this calculation we follow the lead of Conger and Giles (5), who studied the effect on buds of the plant *Tradescantia* of the neutron beam at Oak Ridge, Tennessee. If the fast neutrons and gamma rays arising in the nuclear reactor are excluded from the patient by shielding, then atomic ionization and its associated tissue destruction occur only when the thermal neutrons are captured. Radiations arise from every atom at the time of capture, and in addition a few capture reactions result in the formation of a radioactive daughter isotope, the continuing energy from which may be computed.

The energy of ionization per unit time per unit volume of tissue for each element therein =

$$F \times N \times \sigma_a \times E \times A$$

in which F = flux, number of thermal neutrons crossing a cm.^{2} per second, N = number of atoms of an element per unit volume; σ_a = the capture or absorption cross section, the tendency to capture expressed as the apparent area in cm.^{2} presented by a single nucleus of the element to the beam of slow neutrons; E = energy in million electron volts of radiations emitted after the capture of the neutron; A = the fraction of this emitted energy absorbed in the tissue.

To convert electron volts into roentgen equivalent physical per minute (rep/min.), the factor $r = 5.22 \times 10^{13}$ ev. was used, so that when all values are expressed in the proper units, we have:

Equation I: rep/min.

$$= \frac{(F \times 60)N\sigma_a(E \times 10^6)A}{5.22 \times 10^{13}}.$$

Our source for each of the values was as follows:

F: to facilitate comparison of our data for the brain with those of Conger and Giles in plants, we have used their figure for flux of thermal neutrons $= 1.25 \times 10^9$ n_{th}/cm.^{2}/sec.; N = the number of atoms/cc.; the concentration of each of the elements in normal and neoplastic brain was given to us by Dr. J. Folch-pi largely from his personal analysis of both types of tissue (6).

σ_a: the isotopic capture cross sections, with the exception of that for B^{10} taken from the later compilation of Sullivan, are from Nuclear Data by Way, Fano, Scott, and Thew (7).

A: all of the energy from electrons, protons and alpha particles is absorbed in the brain, so that in these cases A = 1. This is not true, however, for gamma rays.

In order to arrive at a first approximation of the fraction of gamma ray energy absorbed in the radiated material, an assumption as to the geometry of the neutron distribution is required. Because of the fairly rapid absorption of thermal neutrons in tissue or water, the flux will drop rapidly as neutrons diffuse into the brain. This tendency is measured in terms of the diffusion length of neutrons, defined as that distance required to reduce the flux of a plane infinite beam of neutrons to 1/e of its original value. This figure for thermal neutrons in water is 2.85 cm. The absorption is somewhat greater in tissue, especially in tissue containing B^{10}. We have not attempted to calculate a corrected diffusion length for slow neutrons in brain, but experimental measurements of this datum in phantoms are being undertaken. This correction is probably not important for the computation here because, as will be seen in the table and further discussion, the total gamma ray energy is of small significance in comparison with that from the heavy particles.

The percentage of the energy absorbed from the gamma radiation may be calculated on the basis of the following model. A sphere of radius equal to the diffusion length of thermal neutrons in water (2.85 cm.) is assumed to be uniformly irradiated with the production of N gamma rays/cc./min. Let dD represent the contribution to the radiation dosage at the center of the sphere resulting from the gamma rays originating in the volume element enclosed between the radial shells at r and r + dr of Figure 2.

TABLE I

Neutron radiation of brain and glioma

rep/minute of ionization absorbed in normal brain and tumor due to capture reactions of each of the elements present plus boron given i.v.

Element	Atomic number	Atomic weight	Per cent of net weight			N No. of atoms/cc. × 10^{24}			σ_a Capture cross section × 10^{-24} cm^2	E Capture radiation emitted and energy		A Fraction of energy absorbed in tissue	Rep/min. absorbed in tissue		
			Gray matter	White matter	Tumor	Gray matter	White matter	Tumor		Type	Energy (mev.)		Gray matter	White matter	Tumor
H	1	1.0078	10.8	11.1	10.8	.0645	0.0663	.0645	.32	γ	2.23	.071	4.72	4.86	4.72
C	6	12.00	10.0	20.0	10.0	.00502	.0104	.00502	.0047	γ	5.0	.051	.0087	.0180	.0087
N	7	14.008	1.74	1.70	2.20 (maximum)	.000748	.000731	.000945	1.7	p^+	.6	1.0	1.10	1.08	1.39
O	8	16.00	76.5	65.9	76.0	.0288	.0248	.0286	.0009	γ	>2	.076	.0057	.0049	.0057
Na	11	22.997	.116	.107	.116	.0000304	.0000280	.0000304	.45	γ	>1	.091	.0018	.0017	.0018
Mg	12	24.32	.013	.013	.013	.0000032	.0000032	.0000032	.07	γ	>1	.091	.00003	.00003	.00003
P	15	31.02	.210	.390	.248	.0000407	.0000737	.0000482	.15	γ	~1.25	.086	.00076	.00141	.00090
S	16	32.06	.050	.200	.040	.0000094	.0000376	.0000075	.5	γ	>1	.091	.00062	.00247	.00049
Cl	17	35.457	.128	.128	.128	.0000217	.0000217	.0000217	32.5	γ	~2.85	.066	0.191	0.191	0.191
K	19	39.10	.374	.374	.374	.0000576	.0000576	.0000576	2.05	γ	>1	.091	.0155	.0155	.0155
Ca	20	40.08	.004	.004	.004	.0000006	.0000006	.0000006	.42	γ	>1	.091	.00003	.00003	.00003
B^{10}	5	10.00	.0015	.0010	.0050	.00000090	.00000060	.00000301	3900.	α^{++}	2.40	1.0	12.20	8.11	40.73
Rep/min. from radioactive daughters															
Na^{23}			.116	.107	.116	.000304	.0000280	.0000304	.45	β	.47 average	1.0	.0093	.0085	.0093
										γ	4.13	.057	.0047	.0043	.0047
P^{31}			.210	.390	.248	.0000407	.0000757	.0000482	.15	β	.57 average	1.0	.0050	.0094	.0060
Cl^{37}			.0315	.0315	.0315	.0000051	.0000051	.0000051	.6	β	1.1 average	1.0	.0048	.0048	.0048
										γ	1.9 average	.077	.00013	.00012	.00012
Total rep/min. absorbed													18.27	14.31	47.09
Per cent of total rep/min. due to B^{10}													66.8%	56.7%	86.5%

F = Thermal neutron flux = $1.25 \times 10^8 n_{th}/cm.^2/sec.$

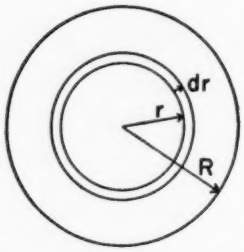

FIG. 2. PERCENTAGE ABSORPTION OF ENERGY OF GAMMA RADIATION

Then:

$$dD = (k)(N)\frac{(e^{-\mu r})}{r^2}(4\pi r^2\, dr)\ \text{rep/min.}$$

In this equation, k represents the radiation dosage rate in rep/min. at a distance of 1 cm. from a gamma ray source of 1 disintegration/min., and is a constant for a given gamma ray energy. N is the number of gamma rays/cc./min. in the source material, and $(e^{-\mu r}/r^2)$ is the attenuation of the gamma rays at a distance r in cm. from the point of their source. The absorption coefficient includes the photoelectric effect, the effect of Compton absorption, and pair production, and is a function only of the energy.

X-rays and gamma rays are absorbed by matter in three different ways: *1*) When the minute mass associated with an X-ray or gamma ray (quantum or photon) collides with an atom it may transfer its entire energy to an orbital electron, ejecting it from the atom. This process is known as the photoelectric effect. A wave strikes and an electron emerges. *2*) A gamma ray photon of shorter wave length, *i.e.*, with higher energies, is more likely to retain some of its energy and bounce off the orbital electron sending it off at one angle while the photon moves off at another angle—a billiard ball type of collision—in which the scattered photon, after the collision, has a longer wave length, less energy and a different direction from the incident photon. This is the Compton effect. *3*) A photon at a still shorter wave length and higher energy above 1.02 mev. may, when it passes near a nucleus, lose its wave characteristic and abruptly materialize into two particles—an electron and a positron. This is known as pair production.

$(4\pi r^2\, dr)$ is the volume of the differential element enclosed in the radial shells at r and r + dr. If the equation is integrated from 0 to R, the total dose rate at the center of the sphere of radius R will be found.

$$D_R = \frac{4\pi kN}{\mu}(1 - e^{-\mu R}).$$

The dosage rate of a sphere of infinite radius, *i.e.*, one in which all of the liberated energy would be absorbed, would be:

$$D_\infty = \frac{4\pi kN}{\mu}$$

and the ratio is:

Equation II: $$\frac{D_R}{D_\infty} = (1 - e^{-\mu R}) = A.$$

This ratio is important because it gives an approximation to the ratio of the energy absorbed per cc. of medium, D_R, to the energy liberated per cc., D_∞. This is identical to the constant A in Equation I.

μ is determined primarily by the Compton absorption coefficient as calculated from the Klein-Nishina (8) equations. A convenient graph from which values of μ in water can be read off directly has been published by Marinelli, Quimby, and Hine (9) (R = 2.85 cm.). In the present case where μR is much less than 1, A can be simply written as $A = \mu R$. It is obvious that these calculations give only a rough estimate of the contribution of the gamma ray to the total dose.

Using the foregoing data and method, we have computed the roentgen equivalent physical/min. arising in normal brain and in neoplasm as a consequence of slow neutron capture for the naturally occurring elements H, C, N, O, Na, Mg, P, S, Cl, K, and Ca and for boron.[10] The results are recorded in Table I.[4]

We have injected only 5 g. of sodium borate into patients during operations on their gliomas, obtaining data of the order of magnitude recorded in Figure 1. However, when the patient is irradiated at the nuclear reactor for therapy, Dr. Farr and his collaborators give intravenously about 15 g. of sodium borate/70 Kg. of body weight so that levels about 3 × those recorded in Figure 1, or 50 γ of B^{10}/g. of tumor and 15 γ of B^{10}/g. of normal gray matter during the first 30 minutes af-

[4] If the conversion factor 5.9×10^{13} instead of 5.22×10^{13} is used to convert electron volts into roentgen equivalent physical per minute (rep/min.) we arrive at values of total rep/min. absorbed in gray matter as 16.17, in white matter as 12.66, and in tumor as 41.67.

ter injection, have been selected as the values on which to base the computation. It will be noted that the estimate is above .02 rep/min. only for H, N, and Cl. All of the remaining eight elements normally present add a total of only .05 rep/min. In addition, we give our computation for the ionization arising from the formation of radioactive Cl^{38} from the Cl^{37} constituting 24.67% of the normally occurring Cl, since we conclude that this gives rise to an inconsequential amount of radiation—a marked divergence from the opinion expressed by Conger and Giles, who estimate .1 — .52 rep/min. in their plants, which have only ¼ the Cl concentration of the human brain. Our result is based on these figures for Cl^{38} decay:

β^- 5.2 mev. 53%, 1.2 mev. 36%, 2.7 mev. 11%, giving an average maximum energy of 3.49 mev. resulting in an average energy formation of 1.16 mev. per disintegration. (A for β^- particles was taken as 1.)

γ 1.60 mev. 43%, 2.15 mev. 57%—average 1.91 mev.

We computed A from $\mu R = .023 \times 2.85 = .066$ for γ rays. Substituting these figures in Equation I, we calculate only .0049 rep/min. arising from Cl^{38} decay in our material, and even this small figure assumes that the biologic effect from this radiation at half life 37 min. would be the same as though it were all given off at once. In subsequent correspondence with Dr. Conger, we learn that the capture cross section of 40 for naturally occurring Cl was used by him instead of the value 0.6 for Cl^{37} which has since become available and which is, of course, the figure applicable to the formation of Cl^{38}. This accounts for much of the discrepancy between the two calculations. The results of similar calculations based on our same assumptions for Na^{24} and P^{32} are also charted in Table I and show that no significant quantities ensue from these sources. A further delayed radiation comes from the C^{14} yielded by the reaction $N^{14} + n = [N^{15}] = C^{14} + p^+$, but the 5,700 year half life of this weak (.155 mev.) β^- emitter precludes an observable biologic effect under these circumstances over a 50 year period in man.

The figures in Table I show that for the concentrations of B^{10} indicated in normal and neoplastic brain, the site of this isotope largely determines the site of the rep/min. Further factors contribute to the conclusion that the B^{10} will be the main cause of radiation damage, to wit, that heavy particles are much more efficient than γ rays in their biological effect on chromosomes. Kotval and Gray (1947), cited by Conger and Giles, found alpha particles 7.8 times as efficient as X-rays per unit dose measured physically in producing isochromatid deletions, and the same particles were 4.1 times as effective as X-rays in producing chromatid plus isochromatid deletions.[5]

Conger and Giles have demonstrated a further increase in efficiency when the alpha radiation arises from within the tissue of 14:1 for isochromatid deletions and 11:1 for chromatid plus isochromatid deletions. The chief disturbing factor in normal tissue, aside from the B^{10} there, is the gamma ray evolving when hydrogen captures a neutron, which has the same lesser efficiency as X-rays in causing cellular death.

If we multiply a factor of roughly 6 of Kotval and Gray for the increased efficiency of heavy particles over X- and gamma rays, by 12, the additional increase in efficiency noted by Conger and Giles for alpha radiation arising within tissue, we might conclude that the rep/min. arising from the disintegration of boron would be about 70 times as destructive as the γ ray from hydrogen. However, one of the major reasons suggested for the increased efficiency of the internal particulate radiation in the lily bulbs of Conger and Giles is the concentration of the boron in the nucleus and chromosomes as compared with the cytoplasm. Hence an increased number of chromosome "hits" per unit dose is more likely from the intracellular and intranuclear boron. Since within 30 minutes after intravenous injection of the borax in man it seems unlikely that much of it has penetrated into the cell, much less the nucleus, we have thought it appropriate to estimate conservatively the greater efficiency of particulate radiation in our material

[5] A "chromatid" is one of the two sister structures formed by the longitudinal splitting of a chromosome during mitosis. Irradiation at a stage in which the chromosomes are split may produce a break in only one of the members of a pair of chromatids. Removal of a portion of the chromatid at the breakage point will tend to prevent reunion of the broken ends and a chromatid deletion is said to have occurred. If both chromatids of the pair are broken at the same level and reunion does not occur, the aberration is termed an isochromatid deletion.

TABLE II

Element	Rep/min. absorbed in tissue × RBE		
	Gray matter	White matter	Glioma
N	5.5	5.4	7.0
B^{10}	61.0	40.6	203.7
All other elements in Table I	5.0	5.1	5.0
Totals	71.5	51.1	215.7
Per cent of radiation effect in tissue due to B^{10}	85%	79%	94%

It is apparent that at these concentrations the site of the boron largely determines the radiation effect, and that when the concentration in tumor is 3.3× that in gray matter (50γ:15γ), the destructive effect in tumor may perhaps be expected to be 3× that in the gray matter.

and to place it at about 5 × that of the gamma rays.

Accordingly we use a factor for relative biologic efficiency (RBE) of 5 for particulate radiation and our tentative estimate of the radiation effect in tissue is based on the factor rep/min. × RBE. This factor we may call roentgen equivalent biological/min.; we use this more general term in preference to Parker's roentgen equivalent mammal/min. (10) because most of the data on which the extrapolation is based are derived from plants. If then we multiply by 5 the rep/min. arising from N and B^{10} and substitute this for the values in the last three columns of Table I we emerge with the estimate of effect on tissue indicated in Table II.

SUMMARY AND CONCLUSIONS

Data are presented to show the degree to which intravenously injected borax concentrates in malignant brain tumors and in normal tissues including brain. On the basis of these data for boron and those from other sources for other elements we have computed to a first approximation the amount of biologic radiation damage (relative biologic efficiency × rep/min.) arising from each element consequent upon exposure to a beam of slow neutrons. The figures reveal that: *1*) for the expected concentrations in tissue following injection of borax, the locus of the boron atom would be the principal determinant of the site of damage; and *2*) at the readily achieved differential concentrations between glioma and brain of 3.3:1, the radiation damage in tumor would be about 3 × that in normal brain—if one assumes uniform distribution of the neutron flux.

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PROCEEDINGS OF THE FORTY-FOURTH ANNUAL MEETING OF THE AMERICAN SOCIETY FOR CLINICAL INVESTIGATION HELD IN ATLANTIC CITY, N. J., MAY 5, 1952

PRESIDENTIAL ADDRESS

THE "LOGARITHMIC PHASE" OF MEDICAL PROGRESS

BY W. BARRY WOOD, JR.

By custom the presiding officer of this society is expected to open the scientific session with a presidential address. This obligation, which I accept with grave misgivings, I shall try to fulfill in as short a time as possible, so that we may proceed promptly to the main program.

The thesis which I should like to present briefly, is that today we find ourselves in, or rapidly approaching, the "logarithmic phase" of medical progress.

The choice of my crude analogy is, of course, conditioned by my own past training and interests. Reference to the bacterial growth curve may be justified, however, on the grounds that it is familiar to nearly everyone in medicine (Figure 1). You will recall that the logarithm

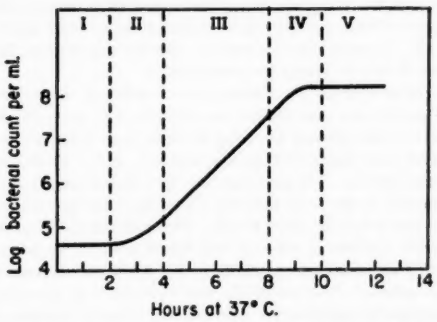


FIG. 1. CONVENTIONALIZED CURVE OF BACTERIAL GROWTH

of the number of viable organisms in a bacterial culture is customarily plotted on the vertical axis and the duration of incubation (in hours) on the horizontal. When cultural conditions are optimal, the curve exhibits five more or less separate phases of growth.

The first phase, usually referred to as the *lag phase*, is characterized by a stationary population. To consider it a latent period in the growth cycle is, as I shall explain in a moment, incorrect. The second period represents the transition from a stable population to one of rapid multiplication. The relatively gradual acceleration of multiplication in this *transitional period* is attributed to the failure of all of the cells to start multiplying at exactly the same moment. During the third phase of growth, the familiar *logarithmic phase*, all viable cells are presumably dividing at a maximum rate, causing the population of the culture to increase exponentially. Phases IV and V represent the final deceleration of growth, ending in a relatively stable population.

Today we need not consider the last two phases of the curve except to recall that premature cessation of bacterial growth may be caused by adverse cultural conditions, as well as by the addition of antimicrobial agents which interfere with the metabolism of dividing cells. The analogy here is obvious to anyone who has contemplated the possible effects on medical science of atomic warfare, of a sudden collapse or further disorganization of world economy, of an emphasis upon quantity instead of quality in medical education, of a sudden drying up of sources of research funds, or of a requirement for "responsible investigators" to prepare more progress reports than we are already required to do. Any one of these and a host of other unpleasant eventualities might cause a significant deceleration of medical progress even within our own lifetimes.

I should like to suggest that we turn our backs on these disturbing thoughts and return for a moment to the past, or, in other words, to the first phase of the growth curve. As I have already stated, the lag phase is not in reality a latent period. On the contrary, there is good evidence that individual cells in bacterial cultures are metabolically more active late in the lag phase than in any other phase of the cycle. This accelerated metabolism, unassociated with rapid cell division, causes two significant changes to take place in the culture. First, it results in an increased protoplasmic mass of individual cells; the oversized or giant organisms of the late lag phase are thus formed (Figure 2). Secondly, it brings about important alterations in the chemical environment of each organism—changes essential to the process of multiplication.

The counterparts of these two characteristics of the lag phase are not difficult to find in the early history of medicine. Viewed from our present vantage point on the steeply sloping curve of modern achievement, the era from Hippocrates to Claude Bernard may appear to have been a latent period, but, like the lag phase of bacterial growth, it teemed with ferment, particularly during its later decades. This ferment likewise gave rise to giants—Harvey, Leeuwenhoek, John Hunter, Lavoisier, and Liebig, to mention but a few. Like the oversized bacterial cells of the lag phase, these great figures of the past exerted a profound influence upon their environments, an influence which paved the way for the "logarithmic

phase" of scientific progress, in which we now find ourselves.

The century of medicine between Claude Bernard and the present was one of rapid acceleration, quite analogous to the steepening slope of phase II of the growth curve. Just as the oversized bacteria of the lag phase are replaced by the smaller rapidly dividing cells which characterize the logarithmic phase, so have the medical giants of the late nineteenth and early twentieth centuries given way to a host of well-trained scientists, educators, and physicians, individually of lesser stature but collectively of far greater potential. As the standards of medicine have risen, the prominence of the few has become relatively dwarfed by the accomplishments of the many.

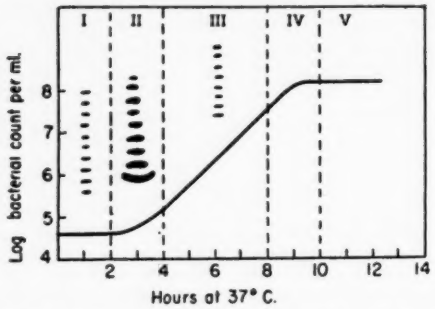


FIG. 2. DIAGRAM INDICATING CHANGES THAT OCCUR IN THE SIZE OF THE BACTERIAL CELLS DURING THE FIRST THREE PHASES OF THE GROWTH CURVE

Whether or not, in 1952, we have truly reached the "logarithmic phase" of medical progress may certainly be debated. It may be argued that progress will continue to accelerate for years or even decades before it enters an exponential phase. No one can deny, however, that the curve upon which we now find ourselves at the mid-century has a far steeper slope than that along which the founders of this society were moving more than forty years ago. The increase in slope has brought profound changes upon literally every phase of medicine. We would do well to examine briefly a few of their more striking effects upon us as physicians, teachers, and investigators.

First as physicians, engaged in the care of ill patients, we are keenly aware of our inability to keep abreast of even the most important advances in clinical medicine. When we scan the contents of medical journals, we are appalled by the volume of new information with which we should be familiar. To meet this problem, which every day is becoming more difficult, we have had only one recourse—namely, to specialize. No matter how much the trend toward specialization is decried by the Academy of General Practice, by medical economists, by educators, or by the lay public, it is here to stay, for it is an inevitable result of the steepness of the progress curve. The era when a single doctor could be an expert in all of medicine, or even in all of internal medicine, has long since passed. To bring to his patients the best that medicine has to offer, the physician of today must limit the scope of his practice to that field in which he is able to *remain* competently trained.[1] If his acquisition of new knowledge ends with his completion of formal schooling or his receipt of a certificate from a specialty board, he is doomed to an ignominious fate of professional obsolescence.

Secondly, the accelerated tempo of medical progress has affected medical education fully as much as it has influenced clinical practice. Only too well does the present generation of students and teachers realize that what is taught today may be outmoded tomorrow. The steepening curve of progress not only causes textbooks to become obsolete with discouraging promptness, but also renders senior faculty members prematurely senile in the eyes of their younger colleagues. Refined laboratory methods have increased the accuracy of present day diagnoses far beyond the dreams of the most skillful elders of the past. These precise diagnostic tools, as well as the numerous specific agents of modern therapy, require for their proper use a more intimate knowledge of chemistry, physics, and pathologic physiology than was ever before considered relevant to clinical medicine. So vast has this body of pertinent preclinical information become that no one teacher of medicine can possibly master it all. To meet the situation, he, like the practitioner, has been forced to resort to specialization.

Let us contrast for a moment a department of medicine of today with one of four or five decades ago. At the turn of the century teaching services were usually composed of a single professor, a resident, and a handful of house officers. The professor was met ceremoniously each morning at the front door by his resident and escorted to the wards for the daily round. There, followed by an attentive retinue of students and house officers, he passed final judgment on every manner of problem encountered at the bedside. Because of his vast experience in the management of patients, his opinions were rarely questioned, and his orders were followed to the letter.

How, I wonder, would such a versatile soloist of the clinic have viewed his counterpart of the mid-twentieth century? The modern professor of medicine, whatever else he may be, is certainly no soloist. To meet the requirements of progressive teaching, he has gathered about him a galaxy of highly trained colleagues, whose combined knowledge he hopes will cover the rapidly expanding fields of internal medicine. No longer a virtuoso, he has become the conductor of an orchestra composed of experts in an ever-increasing number of sub-

[1] By this statement I do not imply that internists should practice only subspecialties. Quite to the contrary, I believe that every physician should be broadly trained and should strive to maintain his competence in as much of general medicine as possible. At the same time, aware of his deficiencies, he should seek the aid of an expert consultant whenever his own ignorance may jeopardize the welfare of a patient.

specialties. Weekly at grand rounds, before an audience of students, house officers, and staff, he is expected to conduct a concert in which he displays the talents of his various experts by allowing them in turn to carry the melody. For any discord that results he is held personally responsible. In some respects, his assignment might frighten even Toscanini, for just as the orchestra is trained to perfection, one of its talented members will suddenly introduce a new instrument—a longer, more versatile catheter, an artificial kidney, another triumph of chemotherapy. With each innovation the professor is expected to become quickly familiar, for as conductor he must realize its possibilities and limitations, in order to integrate it with the rest of the orchestra. Judging from my own brief experience in this exacting role, I wonder if the average professor of medicine today ever really "knows the score."

Finally, the character of medical research has been changed by the rapidity of scientific advance. As the body of knowledge available to medical investigators has increased, so has the need for specialization in research become mandatory. Team projects are the fashion of the day. To be sure, many problems can be solved only by collaboration of experts from a variety of fields. But as every scientist knows, ideas come from individuals, not from groups or institutes. Again the giants of the recent past—Claude Bernard, Pasteur, Emil Fischer, and Ehrlich, each of whose contributions to medical science cover a wide range of subjects—have been succeeded by men whose research has perforce been limited to a lesser scope. Today, more than ever before, individual workers must concentrate their efforts on a single, relatively narrow field, in order to contribute significantly to medical knowledge. To dig deeply at the frontiers of modern medicine requires decades rather than days, the whole life of the investigator rather than the term of his research grant.

To its medical scientists, contemporary society has been most generous. Well has it heeded the advice of Pasteur: "Take interest, I implore you, in those sacred dwellings which one designates by the expressive term, laboratories. Demand that they be multiplied, that they be adorned. These are the temples of the future."

The scientific program, which we are about to hear, and the large number of equally excellent reports which I was forced to omit from the final program, provide convincing evidence that the clinical investigator of today, in spite of daily distractions of practice, teaching, and administration, is making good use of his laboratories. The variety and content of the individual papers will, I am sure, eloquently support the general thesis that the slope of the medical progress curve as of 1952 is indeed steep.

ABSTRACTS

Body Potassium Deficiency in Diabetes Mellitus. J. K. AIKAWA, J. H. FELTS and G. T. HARRELL, JR.,* Winston-Salem, N. C.

The body content of exchangeable potassium (K_e) was measured by the radioisotope (K^{42}) dilution technique in 42 diabetic patients, 22 females and 20 males, age range —18 to 78 years. Patients were hospitalized for regulation, for treatment of complications, or for therapy of unrelated diseases. The diabetes varied widely in severity, duration and adequacy of control; 2 patients were in acidosis and coma. All but one received insulin.

The mean initial K_e value in the females was 27.3 ± s.d. 9.77 meq./kg., with a range between 12.9 and 50.5 meq./kg.; the normal value in young females is 31.5 meq./kg. In 20 males, the mean was 31.4 ± s.d. 7.25 meq./kg., with a range between 16.6 and 53.4 meq./kg., compared with a normal of 46.3 meq./kg.

In 20 subjects, fifty serial determinations were performed, at weekly intervals when possible, in an attempt to determine whether the K_e would change. In 15 instances, following the initial determination, an oral supplement of KCl, 3 gm. daily, was given; 6 of these retained potassium. Two of 5 patients not given a supplement retained dietary potassium as control of the diabetes improved or as other disease processes were corrected. No change was noted in K_e in the remaining 12 patients. All serum potassium values throughout the study were within normal limits (3.5 to 5.0 meq./kg.) as determined by flame photometry. It was not always possible to predict from the initial K_e value, the state of regulation, or the intercurrent disease whether the patient would retain potassium.

These data suggest that a body potassium deficit may exist, not only in patients with diabetic acidosis and coma, but also in those diabetics without clinical, chemical or electrocardiographic evidence of such a deficiency.

Triethylene Melamine in Malignant Disease. LOUIS K. ALPERT, Washington, D. C. (Introduced by Theodore J. Abernethy).

Fifty-two patients with various malignant diseases, predominantly lymphomas, were treated with Triethylene melamine (TEM) by oral administration. In approximately two-thirds of the patients with lymphomatous disorders, the response was good or excellent, as manifested by a major degree of regression of lymph node masses or return of hematologic status toward normal, with pronounced symptomatic improvement. The patients with lymphosarcoma or chronic lymphatic leukemia were more readily controlled than those with Hodgkin's disease or chronic myeloid leukemia. Those who failed to improve were in the late stages of their disease when therapy was started.

In the acute leukemias, the number of circulating white cells was reduced, but the toxic features of the disease were not altered, and in some instances it appeared that TEM may have had a deleterious effect. No objective improvement was noted in the patients with fibrosarcomas or carcinomas of the lung, stomach, thyroid or colon.

The sensitivity of the lymphomas to TEM in the individual patients was unpredictable, as indicated by the wide variation in the total dosage, of from 5 to 200 mgm., required to produce a maximum effect. Hence, the therapy could not be standardized, but was determined by an intermittent and gradually increasing schedule, over periods ranging from one to twenty-three weeks.

Hematopoietic depression was the limiting factor in the total amount of TEM which could be administered, particularly to patients with a high degree of "activity" of their disease.

The demonstrated effectiveness of Triethylene melamine in the therapy of lymphomas brings it within the current concept of the management of malignant disease by agents which will permit the temporary return of a significant percentage of patients to a normal manner of living, even though permanent eradication of the disease is not produced.

Study of Left Atrial Pressure Waves in Relation to Pulmonary "Capillary" Pressure Waves in Patients with Mitral Stenosis. WILLIAM H. AMES, ERNEST O. THEILEN, JOHANN L. EHRENHAFT and JAMES W. CULBERTSON, Iowa City, Iowa. (Introduced by William B. Bean).

Left atrial pressure waves have been recorded at operation for mitral valvuloplasty in seven patients by means of a catheter inserted through the auricular appendage and connected to an electromanometer. Pulmonary "capillary" pressure waves likewise have been registered preoperatively in ten patients. These two types of wave forms were available for direct comparison in four of the patients. There was good positive correlation between left atrial and pulmonary "capillary" waves with respect to both pressure levels and wave contours. Left atrial mean pressures were equal to or greater than the pulmonary "capillary" mean pressures. Patients with normal sinus rhythm exhibited two distinct waves in each location, the first following the onset of electrical systole of the atrium and the second following the onset of electrical systole of the ventricle. Analysis of this second wave yielded clinically useful information concerning the degree of mitral regurgitation, even in the presence of auricular fibrillation.

Planimetric measurements of corresponding left atrial and pulmonary "capillary" regurgitant pulse wave areas were in close agreement. In the four patients the left atrial waves varied considerably, with mean values of 5.2

* Member.

to 9.4 mm Hg, and pulmonary "capillary" waves were proportionately lower (3.3 to 7.9). These values must be distinguished from the mean left atrial and pulmonary "capillary" blood pressure levels, which ranged from 24 to 34 and 17 to 24 mm Hg respectively. All records were analyzed thus and the results found to agree with clinical impressions made by physical examination previously and by intra-atrial palpation at operation.

These observations indicate that pulmonary "capillary" pressure recording may provide helpful information for the evaluation of patients prior to mitral valvuloplasty and for the assessment of postoperative results by objective physiological methods.

Minor Respiratory Diseases: Studies of MR-3 Virus Disease in Human Volunteers. LEON T. ATLAS, Boston, Massachusetts. (Introduced by John G. Gibson 2nd).

Nasal washings were collected from a donor early after onset of respiratory symptoms. His nasal washings and nasal washings from volunteers infected by his secretions were inoculated intra-nasally into 133 volunteers kept in isolation. Third to fourth passage allantoic fluids of embryonated eggs inoculated with the donor's nasal washings were instilled into 47 volunteers.

It was found that both nasal washings and egg cultivated virus produced a consistent and specific disease entity in volunteers, whose illnesses recapitulated the signs and symptoms suffered by the donor. Volunteers receiving normal control inocula had no such disease. The signs and symptoms responsible for the observers' subjective judgment of severity of disease or no disease were determined by statistical analysis of the recorded data. Following a three to five day incubation time, two out of three of running nose, coughing, and chest pain developed in all men considered as definitely sick. A profuse rhinitis was observed in all moderate or severe infections. In addition, conjunctivitis, headache, malaise, muscle and joint aches, fever, and leucopenia were often present. Some volunteers developed lingering weakness, persistent coughing and chest pains resembling those of acute benign pericarditis.

Infection by *MR-3* virus produced a significant resistance to homologous reinfection for 8 months. However, 9 of 22 men upon homologous reinoculation developed an atypical disease characterized by minimal upper respiratory signs but one or more of malaise, fever, coughing, chest pain and migratory polyarthralgia. Recovery from *MR-3* infection did not prevent infection by *MR-1* or *MR-4* viruses. Transmission of *MR-3* disease from infected to contact volunteers was demonstrated. Cultivated fluids beyond the fifth egg passage produced disease so attenuated in incidence and severity that objective evaluation of clinical findings was no longer practical.

The Relation of Colonial Morphology and Antigenic Structure to Virulence in Pneumococcus. ROBERT AUSTRIAN, Baltimore, Md. (Introduced by Palmer H. Futcher).

Attempts to relate colonial morphology, antigenic structure and virulence in a variety of bacterial species have resulted, at times, in apparent inconsistencies. A study of these bacterial properties in pneumococcus employing selective cultural and serologic techniques and transformation reactions has afforded a better understanding of their interrelationships.

With the definition of the rough encapsulated variant in the experiments reported here, four distinct colonial forms of pneumococcus may now be recognized: smooth encapsulated, rough encapsulated, smooth unencapsulated, and rough unencapsulated. A difference between the smooth and rough variants appears to reside in the mode of separation of their cells after division. Cells of smooth variants separate into pairs or short chains after division whereas those of rough forms remain attached forming chains of great length. This characteristic of rough variants is responsible apparently for the irregular surfaces and margins of their colonies on solid media and is manifested both by encapsulated and unencapsulated forms.

Antigenic analysis of smooth and of rough unencapsulated pneumococci reveals both to possess species-specific C carbohydrate and type-specific M protein but both are phagocyted readily by leukocytes and lack virulence for mice. Encapsulated smooth and rough organisms produce, in addition to the antigens mentioned, type-specific capsular polysaccharide, are not phagocyted readily in the absence of homologous anticapsular antibody and are virulent for mice.

Studies of colonial variation in pneumococcal transformation reactions demonstrate that the genetic determinants controlling encapsulation and the mode of cell separation are inherited independently of one another. These experiments show clearly that the principal factor in the virulence of pneumococcus is the presence or absence of encapsulation regardless of whether the mode of cell separation after division is that of the rough or that of the smooth variant.

Urinary Excretion of Tetrahydrocortisone. BILLY BAGGETT, JOHN H. GLICK, JR. and RALPH A. KINSELLA, JR., St. Louis, Mo. (Introduced by Ralph Kinsella).

Tetrahydrocortisone (pregnane-3α,17α,21-triol-11,20,dione) has been identified as the principal α-ketolic corticoid in the urine of normal adults and healthy pregnant women. Neutral reducing lipids and neutral formaldehydogenic lipids can be extracted in greatly increased amounts following hydrolysis of their urinary conjugates with a bacterial enzyme preparation containing β-glucuronidase, as previously reported. This hydrolysis results in ten to fiftyfold increases in extractable tetrahydrocortisone, in contrast to the increases in extractable Compounds E and F which are three to fourfold. Quantitative estimations of urinary tetrahydrocortisone and Compounds E and F have been made after separation by paper chromatography. Tetrahydrocortisone has been isolated as the pure crystalline compound and identified; the acetate was prepared for further identification. Urinary excretion of tetrahydrocortisone has been found in normal adults to be

from 3 to 6 mg in 24 hours and in pregnant women to be slightly lower than the lowest values obtained for normal adults. Studies are now in progress to determine the relationship between urinary excretion of tetrahydrocortisone and the administration of ACTH and adrenal steroids.

Acute Responses of T_{mPAH} and Glomerular Filtration Rate to Intravenous Lactate in Uremic Patients. EARL S. BARKER, JOHN KAPP CLARK* and ARCHER P. CROSLEY, JR., Philadelphia, Pa.

The recent demonstration of acute increases in T_{mPAH} and glomerular filtration rate (GFR) following intravenous administration of hypertonic sodium lactate in normal subjects might indicate the mechanism of the clinical improvement in uremia said to result from chronic lactate administration. These measurements were therefore made directly in patients with azotemia due to various renal diseases.

Increases in T_{mPAH}, inulin clearance and endogenous creatinine clearance following lactate 6 in severe uremics were negligible considered in absolute terms (i.e. increases of less than 3 mgm./min. in T_{mPAH} and 4 cc./min. in GFR). However, in terms of percentage increase, the changes following lactate were of the same order of magnitude in uremics of different degrees and in normals (about 30% in T_{mPAH} and somewhat less in GFR).

These data indicate that, so far as tubular transport mechanisms involved in PAH excretion are concerned, each unit of remaining function is capable of responding normally and suggest that even severe renal insufficiency in several types of renal disease may be associated with quantitative reduction rather than qualitative change in the particular system tested. Whatever benefit may be derived from lactate treatment by these mechanisms must accrue from very small changes in absolute terms. Nevertheless it is believed that further study is warranted, since these patients are surviving on extremely small margins of renal function and the changes may therefore be clinically important.

Biochemical Abnormalities in Wilson's Disease. A. G. BEARN and HENRY G. KUNKEL,* New York, N. Y.

In view of recent reports describing changes in copper metabolism in Wilson's disease, a special study of 9 patients suffering from this condition was undertaken. Despite wide differences in age, duration of the disease and degree of hepatic and neurological involvement, all patients showed a marked increase in the daily urinary excretion of copper (range 450–1790 gamma/24 hours—normal 0–25 gamma/24 hours). As a corollary to this study, quantitative analyses for 19 amino acids in the urine from 6 of these patients were carried out by Drs. William H. Stein and Stanford Moore using ion exchange columns. In 5 of these cases the excretion of tremendously increased quantities of 13 of the amino acids was observed (up to or more than 30 times normal, in some instances). Ornithine and citrulline, two amino acids not encountered in normal urine, were present in significant amounts. A striking parallelism was found between the copper and amino acid excretion which suggests that a metabolic relationship may exist between these two defects.

Analyses of serum copper showed that the levels were reduced in 8 of the 9 patients (39–79 gamma/100 ml—normal 90–120 gamma/100 ml), a somewhat surprising finding in view of the increased urinary excretion of this metal. Copper is known to be the prosthetic group of certain enzymes and in serum is present largely as an oxidase. A considerable decrease in serum oxidase activity was observed in all patients thus correlating with the low copper levels. The administration of B.A.L. resulted in an increase in the urinary copper excretion in all patients to whom it was given, and in some instances was followed by slight clinical improvement.

Clinical and Metabolic Effects of Bilateral Adrenalectomy in Man. D. M. BERGENSTAL and C. B. HUGGINS, Chicago, Illinois. (Introduced by A. T. Kenyon).

Bilateral simultaneous adrenalectomy has been carried out by us on 35 human cases with two deaths. An adequate hormonal substitution program has been developed to prevent adrenal insufficiency, and in most cases adequate maintenance hormonal substitution has been 25 mg. cortisone twice a day by mouth and three grams of sodium chloride. Patients with metastatic cancer were adrenalectomized. In 7 cases of prostatic cancer which became reactivated after previous antiandrogenic control, adrenalectomy gave relief of bone pain, increased appetite with weight gain, reduction in acid phosphatase when elevated, elevation of alkaline phosphatase, and in many cases produced shrinkage of tumor. There have been two relapses. Cortisone and ACTH did not give sustained beneficial effect in prostatic cancer. Five cases of mammary cancer have shown significant remission characterized by either regression of tumor, decrease in hemothorax or healing of bone lesions. The miscellaneous group of tumors showed no significant alteration in their course after adrenalectomy. It is reasonable to assume that the regressions are due in part to elimination of critical amounts of sex hormones by adrenalectomy.

The eosinophile test was negative after adrenalectomy in response to saline and ACTH, but a greater than 50 per cent decrease was observed in 6 out of 9 patients given adrenalin. The glucose tolerance, insulin tolerance and Kepler water tests were not significantly altered after operation. A study of 17-ketosteroids and dehydroisoandrosterone excretion before and after adrenalectomy has been done. Alterations in excretion of these steroids (in patients without gonads and adrenals) during episodes of stress and after administration of compounds B, E, F and dehydroisoandrosterone have been studied. Three cases of hypertension, as well as the normotensive patients, have stabilized their blood pressures near the pre-operative levels. Two patients with diabetes mellitus showed no change in insulin requirement after adrenalectomy.

Bone as a Sodium and Potassium Reservoir. WILLIAM H. BERGSTROM, Boston, Mass. (Introduced by James L. Gamble).

Bone contains a large fraction (approximately 25%) of the total amount of sodium present in the body. This report describes an investigation of bone sodium and potassium content and of the availability of these cations during acute electrolyte depletion.

We have determined sodium, potassium and chloride in the bones of groups of normal rats at several stages of maturity. Average sodium concentrations, corrected for extracellular fluid, ranged from 110 mEq/Kg in young rats to 180 mEq/Kg in adult rats. Potassium showed an inverse relation to age, falling from 35 mEq/Kg in immature rats to 19 mEq/Kg in mature rats. These values were corrected for potassium calculated to be present in marrow.

Base depletion was accomplished by intraperitoneal dialysis. The procedure resulted in an average loss of 12 mEq of sodium per Kg of body weight. Mature and immature rats sacrificed 48 hours after depletion showed bone sodium values averaging 25 mEq/Kg less than those of the appropriate age controls. Bone potassium was 12 mEq/Kg below its control concentration.

The data indicate that bone acts as a reservoir from which sodium and potassium are drawn during acute base depletion. The magnitude of the changes observed may be illustrated by reference to a 70 Kg man, in whom comparable values would imply a contribution of 300 mEq of sodium and 150 mEq of potassium to the body base economy, assuming the skeleton to comprise 17% of body weight. The relation of these findings to the interpretation of metabolic balance studies will be discussed.

The Distribution and Utilization of Radio-Iron (Fe^{59}) in Protein-Depleted Rats. W. F. BETHARD, R. W. WISSLER, J. S. THOMPSON and M. J. ROBSON, Chicago, Illinois. (Introduced by L. O. Jacobson).

Protein deprivation in young, adult, male rats was followed promptly by marked diminution of hemoglobin formation, as measured by incorporation of radio-iron into erythrocytes. Within two weeks only 4.4% of the intravenous tracer appeared in erythrocytes of protein-depleted animals, as compared to 59.1% in the erythrocytes of controls. Diminished hemoglobin production was sustained for 35 days, after which protein repletion was instituted. Addition of protein to the diet resulted in rapid restoration of hemoglobin formation to normal. The rapidity and degree of alterations in hemoglobin formation could not be satisfactorily quantitated by either peripheral blood counts or blood volume determinations.

At the nadir of protein depletion, radio-iron tissue distribution was determined. The usual chronology of iron metabolism was completely disrupted. Protein-depleted livers of the experimental group accumulated an average of 46.4% of injected tracer, despite the fact that iron is presumably stored as protein conjugate. Bone marrow of this group contained only 5.59% of the radio-iron at 3 hours; and a maximum of 33.6% appeared at 36 hours. In contrast, control animals yielded 61% of the tracer in the bone marrow at 3 hours, after which an exponential disappearance occurred. Normal livers consistently contained 11% of the injected dose. In tissues examined, 78.6% of the radio-iron was recovered in control animals, but only 53.8% could be accounted for in protein-depleted rats.

New quantitative and temporal alterations in hemoglobin formation during protein depletion have been shown using radio-iron tracer techniques. Disruption of normal time–concentration tissue iron relationships has similarly been demonstrated. These results may help explain the anemia associated with chronic infections, malignant neoplasms, and other debilitating diseases.

The Chemical Determination of Lipids in Needle Biopsy Specimens of Liver. BARBARA H. BILLING, DAVID E. HEIN and HAROLD J. CONLON, Cincinnati, Ohio. (Introduced by Leon Schiff).

In the past, estimations of the lipid content of the human liver have been confined to autopsy material. With the development of needle biopsy techniques, liver lipids can now be determined chemically during life. The biopsy specimen (3–8 mg.) is transferred from the needle to a hook and is immediately weighed with a quartz fibre balance at 15 second intervals for 3 minutes so that a correction for water lost by evaporation can be made. The specimen is frozen, dried in a vacuum desiccator, reweighed and then placed in a tube containing 500 μl ethyl alcohol. The tube is sealed and after 48 hours at 60° C, the specimen is dried and reweighed; the amount of fat extracted can then be calculated. Aliquots of the alcohol extract are used for the determination of phospholipids, total fatty acids and iodine number.

In order to show that tissue obtained by needle biopsy gives a valid representation of the whole liver, in respect to lipid content, needle specimens have been obtained from 18 sites in each of 20 livers obtained at autopsy. Values for the lipid content of the specimens showed good agreement and correlated well with the concentration in the entire liver. In contrast to observations made on experimental animals, the quantitative determinations indicated a relatively uniform distribution of lipids in the liver. Comparison of the histological and chemical findings has made it possible to assign quantitative values to the microscopic grading of fat in the liver.

Forty-six routine liver biopsies taken from 40 patients for diagnostic purposes have been analysed chemically as well as microscopically. No correlation between the concentration of lipids in the blood and the liver has been found.

The Effect of Expansion of the Extracellular Fluid Volume in Conditioning the Diuretic Response to Ingested Isotonic Saline Solution. WILLIAM H. BIRCHARD, Framingham, Massachusetts. (Introduced by Maurice B. Strauss).

The long-recognized fact that man exhibits little increase in urine flow following the ingestion of isotonic

saline solution has been considered evidence of a lack of responsiveness to changes in the volume of extracellular fluid.

However, it has been shown that expansion of extracellular fluid volume by the relatively rapid intravenous infusion of isotonic saline produces a water diuresis in the recumbent subject. The following experiments indicate that the ingestion of isotonic saline also produces a diuretic response when there has been prior expansion of the extracellular fluid volume.

Three normal subjects, each seated quietly in a chair, several hours after breakfast, ingested 1 liter of 0.9% sodium chloride solution. During the ensuing three hours urine flow remained below 1 ml./min., and urinary electrolyte concentration rose to 600 milli-osmols/l.

Subsequently, but before 6 P.M., each subject ingested sufficient saline to constitute a load of 6.2 meq./kg. in addition to salt taken with food.

On the following morning weight gains averaged 0.8 kg., ascribable to an expansion of extracellular fluid volume. The subjects again ingested 1 liter of 0.9% saline. There now occurred prompt diureses of approximately one hour's duration averaging 7.5 ml./min., with urine specific gravities falling to 1005 and urinary electrolyte concentrations to 140 milli-osmols/l.

Thus it would appear that the ingestion of salt solution by subjects whose extracellular fluid volume already is expanded produces a type of diuresis characteristic of decreased antidiuretic activity.

Neural Control of Renal Excretion of Sodium in the Dog. WILLIAM D. BLAKE, New Haven, Conn. (Introduced by Dr. C. N. H. Long).

Recently Kaplan and associates have reiterated the viewpoint that renal denervation increases electrolyte excretion independent of increased filtration rate, primarily on the basis of maintaining high solute concentration in the urine. The present studies confirm the main theses that increased filtration rate is unnecessary for increased sodium output on the denervated side, that the effect is most apparent in the fasting or hydropenic animal and that the significant difference between denervated and control kidneys is the much higher concentration of sodium in the urine from the denervated kidney. The intrarenal mechanisms responsible for this difference in concentration are not clear. With this in mind an attempt has been made to develop a pharmacological technique for studying neural control of renal function. Studies have been done on dogs, with and without pentobarbital anesthesia, under varying conditions of water and salt balance. The usual clearance techniques were used to estimate renal function. Drugs used were adrenalin or synthetic epinephrine and hydergine (Sandoz), a combination of dihydrogenated ergot alkaloids. Studies in this laboratory and elsewhere indicate that epinephrine acts directly on the kidney and may well be the mediator of neural impulses. Conversely, hydergine appears to act, in part at least, by blocking nervous stimuli (data to be presented elsewhere). In hydropenic, unanesthetized dogs infusion of small amounts of adrenalin leads to a marked drop in urinary sodium concentration, whereas the administration of hydergine usually has the opposite effect, i.e. sodium concentration increases. These effects are not noted in dogs made hypervolemic by the infusion of "normal" saline. Beside hydropenia neural control of electrolyte excretion may be a factor in emotional stress and pentobarbital anesthesia.

Bone Marrow Studies in Polycythemia. MATTHEW BLOCK and WILLIAM F. BETHARD, Chicago, Illinois. (Introduced by Clayton Loosli).

In 42 of 47 cases of polycythemia vera, including patients with normal or low hemoglobin, hematocrit and erythrocyte values following phlebotomy, phenylhydrazine or spontaneous hemorrhage, the histopathology of the marrow as seen in section, presented a characteristic pattern. The densely cellular marrow had clumps of stem cells and basophilic erythroblasts, little or no iron, and a normal cell distribution, except for slight erythroblastic hyperplasia. In 4 cases marrow sections were suggestive but not diagnostic; and in 1 case the marrow was completely dissimilar. Marrow sections of 25 cases of secondary polycythemia, some of which had hemoglobins as high as 20–24 grams, differed from those seen in the primary cases. However, our experience with secondary polycythemia with extremely high hemoglobin is limited.

Myeloid metaplasia developed in 6 patients; 2 had been treated with P^{32}. Four had extremely cellular marrows and 2 still had active, uncontrolled polycythemia vera. Myelosclerosis was found in 1 terminal patient.

Clinical control (normal peripheral blood, including normal platelet count, and disappearance of abnormal signs and symptoms) following P^{32} in 8 cases, was correlated with a return of the marrow to normal except for a slight residual erythroblastic hyperplasia. Some of these were followed by serial biopsy as long as four years during 3 courses of treatment. Three other cases, which did not respond to the usual dose of P^{32}, had marrows that were unchanged; during this time their hemoglobin, but not platelet count, was controlled by phlebotomies and spontaneous hemorrhages. Two of these three later were well controlled by larger doses of P^{32}; after which their platelet counts returned to normal and their marrows responded similarly to the other 8 patients.

The Oxygen Pressure Gradient from Alveolar Air to Arterial Blood in Patients with Mitral Stenosis. S. GILBERT BLOUNT, JR., MALCOLM C. MCCORD and LEIGHTON L. ANDERSON, Denver, Colorado. (Introduced by Harry Gordon).

Pathological alterations in the alveolar-capillary membrane have been described in patients with mitral stenosis. These findings suggested the possibility of an impediment to oxygen transfer at the alveolar-capillary membrane level in patients with mitral stenosis.

The peripheral arterial oxygen tension was determined by the technique of Riley et al., and the alveolar oxygen

tension was calculated by the method of Lilienthal et al. This permitted a calculation of the alveolar-capillary oxygen pressure gradient. Twenty young healthy persons were studied by these methods to establish the normal values for this altitude. Fifteen patients with mitral stenosis of varying severity were studied in a like manner. All the patients with mitral stenosis were studied by means of cardiac catheterization.

The mean resting alveolar and arterial oxygen tensions in the normal subjects were 80.06 mm. Hg and 74.60 mm. Hg respectively, thus resulting in a mean alveolar-capillary oxygen pressure gradient of 5.46 mm. Hg. Following exercise, the mean values were 86.05 mm. Hg for alveolar oxygen tension and 75.00 mm. Hg for arterial oxygen tension. The alveolar-capillary gradient was thus 11.05 mm. Hg. In contrast, the mean resting alveolar and arterial oxygen tensions in patients with mitral stenosis were 78.33 mm. Hg and 66.04 mm. Hg. The mean alveolar-capillary gradient was 12.29 mm. Hg. After exercise the mean alveolar oxygen tension was 85.02 mm. Hg, and the mean arterial oxygen tension was 68.38 mm. Hg, giving an alveolar-capillary oxygen pressure gradient of 16.64 mm. Hg.

The differences in the alveolar-capillary oxygen pressure gradient in these two groups indicate a physiologic counterpart to the known pathological alterations in the alveolar-capillary membrane in patients with mitral stenosis. The differentiation of the components of the total alveolar-capillary oxygen pressure gradient is considered. The severity of the mitral stenosis as determined clinically and by cardiac catheterization is correlated with the alveolar-capillary oxygen pressure gradient.

The Pressor Action of Atropine in Veratrum Alkaloid (Veriloid®) Induced Hypertension. ALFRED A. BOLOMEY and ROSEMARIE LENEL, Oakland, California. (Introduced by Eaton M. MacKay).

The recent literature would indicate that while atropine effectively abolishes the bradycardia induced by veratrum alkaloids, it fails to influence significantly their hypotensive action. Our recent studies, however, indicate that a remarkable pressor response to atropine occurs frequently in hypertensive patients made acutely hypotensive with Veriloid®.

Forty-five experiments were carried out on 13 hypertensive patients. Veriloid was administered orally or intravenously in doses sufficient to produce marked hypotension and bradycardia. At the height of the response atropine (0.17 to 0.90 mg.) was administered subcutaneously or intravenously. A sustained and significant rise in systolic and diastolic blood pressure was observed in 32 instances with control blood pressures being approached or exceeded in 19. In 30 studies the pulse rate rose simultaneously while in 4 blood pressure rose before pulse rate. In general, blood pressure and pulse rate changes were parallel in magnitude and duration.

Our studies do not lend themselves to an explanation of this atropine effect. A review of the literature indicates that the doses of atropine used were probably not of sufficient magnitude to increase peripheral resistance. The bradycardia may have been profound enough to depress cardiac output, yet, a reversal of such an effect would not seem sufficient to account for the marked pressor phenomenon observed. The doses of Veriloid® used in these studies were usually in excess of the therapeutically recommended dose; yet the same effect was observed in ambulatory patients taking constant and previously well-tolerated amounts.

Since profound hypotension and bradycardia are known to occur suddenly in patients taking constant and usually well-tolerated doses of veratrum alkaloids, it would appear important that hemodynamic studies aimed at elucidation of this mechanism be initiated.

Steroid Diabetes Occurring During Cortisone and Adrenocorticotropin (ACTH) Therapy. JOHN J. BOOKMAN, STANLEY R. DRACHMAN, LOUIS I. SCHAEFER, and DAVID ADLERSBERG, New York, N. Y. (Introduced by Benjamin Kramer).

Among hundreds of patients treated with Cortisone and ACTH for their basic disease five developed the clinical features of steroid diabetes. None presented glycosuria or hypoglycemia prior to the institution of this form of hormonal therapy; in all the signs of diabetes disappeared with the discontinuation of treatment or with reduction of dosage to minimal maintenance levels, and remained so during a follow up period up to one and a half years.

The group as a whole presented all the known features of steroid diabetes although each patient did not exhibit all the features. Doses of insulin up to 70 to 110 units respectively failed to control the glycosuria in two patients. Ketonuria was observed only in one instance. There was a negative nitrogen balance in the one patient so studied, despite a high level of protein intake. The 17 ketosteroids were low prior to and during ACTH therapy in another individual. All patients were predisposed to diabetes; four patients had a familial background of diabetes, the fifth had a transient glycosuria ten years prior to cortisone treatment and a mild but persistent impairment of glucose tolerance one year after cessation of therapy. This indicates that a primary deficiency of insular function was essential for the precipitation of steroid diabetes. Only one patient developed manifest features of diabetes after cortisone therapy; the four others failed to develop diabetes during cortisone but did so during ACTH administration.

It is suggested that impairment of carbohydrate tolerance developing in well established diabetics during infections, burns and other stress situations may represent instances of steroid diabetes superimposed on pancreatic insular insufficiency. The negative nitrogen balance and the resistance to insulin so characteristic of these "episodes" could be easily explained on this basis.

Excretion of Water and Electrolytes in Patients with Essential Hypertension. WILLIAM A. BRODSKY and HARRY N. GRAUBARTH, Louisville, Kentucky. (Introduced by J. Murray Kinsman).

Osmotic diuresis with mannitol loading was produced in six patients with essential hypertension and without previous evidence of cardiac or renal disease. After abstaining from food and drink for 18 hours, the subjects were excreting urine at rates of flow of 0.98–1.68 cc/min/1.73M², with a sub-maximal osmolarities of 607–700 mosm/L. During loading, urine flow increased to as high as 14.5 cc/min, with osmolarity, as low as 375. Minute losses of Na and Cl were 2–2½ times greater than normal, reaching values of 1.8 and 1.4 mEq per minute, respectively. Calculated values of ideal osmotic work amounted to less than one-half that of normal subjects. Clearance of mannitol, at 63.5 cc per minute, was 25 per cent below normal, while that of pAH, 312 cc, was 50 per cent of normal.

The findings of high rates of Na and Cl excretion with low rates of filtration are compatible with deranged activity of the renal tubules. Although the exact site of tubular damage is unknown, the defective concentrating ability implies distal, and defective composition of urinary solutes, proximal tubular dysfunction. The effects of primary reduction of the arterial supply to the kidney are those of polyuria and chloruria (Verney). It is possible that similar renal mechanisms are operative in uncomplicated hypertensive disease.

Radioactive Potassium Distribution in Metabolic Disorders. BELTON A. BURROWS, MILTON M. ASHLEY and JOHN H. SISSON, Boston, Massachusetts. (Introduced by Francis C. Lowell).

We have found the same rate of distribution of radioactive potassium into body potassium in normal subject, before and during cortisone administration, and in patients with a variety of metabolic disorders: Cushing's Syndrome before and after subtotal adrenalectomy, Addison's Disease before and after treatment, uremic acidosis before and after extracorporeal dialysis, hepatic cirrhosis before and after recovery from decompensation, cardiac failure or respiratory acidosis before and after treatment, and severe potassium deficiency before and after replacement therapy. In all instances equilibration of the radioactive potassium with body potassium was virtually complete at the end of twenty-four hours. Many of these patients had serum potassium concentrations outside the normal range which were not associated with demonstrable changes in the rate of potassium transport across the cellular membrane.

Metabolic balance studies in depleted patients showed close agreement between the amount of potassium retained during replacement therapy and the increase in the amount of body potassium with which the isotope equilibrated (exchangeable potassium) even when such increments amounted to one-half the pretreatment "exchangeable potassium" value. Thus the measured "exchangeable potassium" closely corresponds with the metabolically active body potassium irrespective of profound metabolic alterations or the size of the potassium pool.

The "exchangeable potassium," related to body weight, was often decreased in chronic illness by up to one-half the normal values (40–50 mEq/kilo), although many of the patients had persistent hyperpotassemia. These measurements indicate either a reduction of intracellular potassium concentration or total cell mass, or a proportionate increase in body fat and water, or combinations of these factors. Therefore, these observations offer an explanation for the debilitating effects of chronic illness in terms of reduced organ and muscle function or mass per unit of body weight.

Pulmonary Vascular Function in Mitral Stenosis. DOUGLAS CARROL and RICHARD RILEY,* Baltimore, Maryland.

The diffusing capacity of the lungs has been used to evaluate pulmonary capillary function in 26 patients with mitral stenosis. The findings have been correlated with hemodynamic measurements obtained by pulmonary artery catheterization. In addition, the distribution of ventilation and circulation throughout the lungs has been studied. The patients have been classified on the basis of normal or low diffusing capacity and normal or abnormal distribution.

A low diffusing capacity was found in all cases studied during exercise and indicates that capillary function was impaired. The reduction in capillary function may be due to the following mechanisms: loss of capillaries, thickening of capillary of alveolar walls, obstruction or vasoconstriction of small pulmonary arteries reducing the number of capillaries perfused with blood. Correlation with the hemodynamic studies shows that the mean pulmonary artery pressure and the pulmonary resistance are inversely related to the diffusing capacity. These correlations point toward changes in the small pulmonary arteries, since these vessels are of prime importance in determining pulmonary vascular resistance and in controlling blood flow through the capillaries.

In general, the degree of physiological abnormality was best correlated with the severity of exertional dyspnea and was poorly correlated with duration of disease, hemoptysis, pulmonary edema, paroxysmal nocturnal dyspnea or cardiac enlargement. Abnormalities of distribution, frequently found at rest, improved during exercise.

Post-operative studies suggest that abnormalities of capillary function are in part reversible.

On the Relation of Copper to the Metabolism of Iron. G. E. CARTWRIGHT,* C. J. GUBLER, M. E. LAHEY and M. S. CHASE, Salt Lake City, Utah.

Studies in copper-deficient and in iron-deficient swine indicate that copper is intimately concerned in the metabolism of iron.

Swine deficient in copper, like iron-deficient swine, develop a microcytic hypochromic anemia, normoblastic hyperplasia of the bone marrow, hypoferremia and an increase in the iron-binding capacity of the plasma. The hypoferremia occurs early in the course of the deficiency in spite of the administration of 30 mg./kilogram of body weight of iron per day. Within 24 hours after the

administration of copper there is a marked increase in plasma iron.

By the use of radioactive iron it has been possible to demonstrate that in copper-deficient swine, the absorption of iron from the gastrointestinal tract is decreased. Corroborating this finding is the observation that the iron content of the tissues of the copper-deficient swine is decreased. Impairment of iron absorption does not, however, adequately explain the development of anemia in copper-deficient animals since the anemia does not respond to the parenteral administration of large doses of iron. Furthermore, only about 25 per cent of radioiron administered intravenously is utilized for hemoglobin formation.

Swine deficient in iron accumulate copper in the liver in abnormal amounts. Animals deficient in both iron and copper develop anemia much more rapidly than those deficient in either mineral alone.

These results suggest that in copper-deficient swine the ability to metabolize iron properly is impaired and as a consequence of this an anemia, which is morphologically and chemically indistinguishable from iron deficiency anemia, results.

Physiological Effects of Mechanical Exsufflation in Experimental Obstructive Breathing in Human Subjects. REUBEN M. CHERNIACK and CHARLES A. GORDON, New York, N. Y. (Introduced by Alvan L. Barach).

Obstructed breathing in dogs has been shown to produce pulmonary congestion and edema. These changes have been attributed to the development of an increased negative inspiratory intra-pleural pressure. The present study is concerned with the physiological effects of mechanical exsufflation on experimental obstructive breathing in human subjects. Mechanical exsufflation, developed as a means of eliminating bronchial secretions, depends on a gradual inflation of the lungs by a pressure of 40 mm. Hg, terminated abruptly by a swift opening valve, allowing the pressure to rise to the atmosphere in 0.04 seconds. The expiratory flow rates in normal individuals are thereby increased to about 65 per cent of those found in a maximally vigorous cough. When dyspnea is produced in human subjects by breathing through a 4.0 mm. orifice the peripheral venous pressure (a useful indication of intra-pleural pressure) is lowered in inspiration, and the volume flow rates in inspiration and expiration are prolonged. With exsufflation, dyspnea was relieved and the following physiologic effects were noted: (1) inspiratory venous pressure was elevated to the normal level, (2) inspiratory volume flow rates were markedly increased and (3) expiratory volume flow rates were increased at the onset of expiration. The significance of these findings is discussed.

Antirheumatic and Metabolic Effects of Hydrocortisone in Rheumatoid Arthritis. WILLIAM S. CLARK, JOSEPH A. BLAIS and HENRICK O. TONNING, Boston, Mass. (Introduced by Charles L. Short).

Hydrocortisone was administered to six patients with active rheumatoid arthritis, intramuscularly and orally, for four- to twelve-day periods in doses of 150 to 300 mg. per day. Cortisone acetate was also administered to four of the patients under identical conditions for purposes of comparison. Observations were made on the subjective and objective manifestations of the disease, emotional phenomenon, eosinophils, steroid excretion, glucose tolerance, sedimentation rates and synovial fluids. Three of the patients were studied on the metabolic ward with mineral and nitrogen balance determinations. The steroid was administered both in the form of the acetate and free alcohol. Hydrocortisone acetate was found to be a potent antirheumatic steroid in doses that produced no appreciable metabolic affect as measured by balance studies and 17-ketosteroid excretion. Hydrocortisone acetate and hydrocortisone, mgm. for mgm., had less effect on the subjective manifestations than cortisone acetate, inducing less euphoria, although their effects on the subjective manifestations were similar. Hydrocortisone acetate was found to be metabolically less active than cortisone acetate when administered intramuscularly in duplicate experiments. These studies suggest a greater dissociation between antirheumatic and metabolic effects of hydrocortisone acetate than of cortisone acetate, and a qualitative difference in the effects of these two steroids on the psyche.

A Hemorrhagic Disorder Caused by Circulating Anticoagulant in Patients with Disseminated Lupus Erythematosus. C. LOCKARD CONLEY * and ROBERT C. HARTMANN, Baltimore, Maryland.

Two patients with disseminated lupus erythematosus had a peculiar hemorrhagic disorder attributable to a pronounced coagulation defect. Whole blood clotting times were prolonged, in one case exceeding 24 hours, and there was prolongation of the prothrombin times. Plasma concentrations of fibrinogen, prothrombin and accelerator globulin were normal. Impaired coagulation was caused by a circulating anticoagulant which retarded conversion of prothrombin to thrombin even in the presence of tissue extract thromboplastin. As little as one part of the patient's plasma added to 200 parts of normal blood significantly prolonged the clotting time of the latter. Unlike heparin, the anticoagulant did not retard the clotting of plasma by thrombin, and its effect was not overcome by protamine or toluidine blue. The anticoagulant was not dialyzable, was not absorbed on barium sulfate, withstood heating at 65° C for 30 minutes but was inactivated at 80° C. Plasma fibrinolytic activity was normal, and the concentration of tocopherol was not increased. Serum globulin concentration as measured by the Howe technique was normal. Attempts to separate the anticoagulant by ammonium sulfate fractionation and by electrophoresis were unsuccessful. The anticoagulant titer decreased in one patient during treatment with ACTH and cortisone. The plasma of both patients contained an auto-hemagglutinin, and the Coombs test was positive in the one case examined. Anemia was present but there was little evidence of increased blood destruction. Both patients had biologic false positive serologic tests for syphilis and positive cephalin flocculation tests. L.E. cells were demon-

strated in the blood of one patient and the diagnosis was confirmed at autopsy in the other. The condition described is an additional example of a situation in which a prolonged prothrombin time is attributable to a plasma abnormality other than prothrombin deficiency.

Effects of Hexamethonium Bromide upon Hemodynamics of Cerebral and Coronary Circulation in Hypertension. CHARLES W. CRUMPTON and QUILL R. MURPHY, Madison, Wisconsin. (Introduced by William S. Middleton).

Separate studies of the quantitative blood flow through the brain and heart were performed during sustained hypertension and repeated after a significant blood pressure reduction induced by hexamethonium bromide administered parenterally.

Cerebral hemodynamics and oxygen metabolism were studied in six hypertensive subjects in the supine position before and one hour after intramuscular hexamethonium bromide (0.8–1.2 mg./kg.). All subjects exhibited a grade III–IV degree of hypertensive retinopathy (Keith-Wagener classification). Intramuscular C-6 resulted in a 29% reduction in mean arterial blood pressure and a 30% decrease in cerebral vascular resistance without essential change in cerebral blood flow (56 and 57 cc./100 gm./min.). Cerebral oxygen consumption and cerebral arteriovenous oxygen difference did not change significantly during the period of blood pressure reduction.

Studies of the coronary circulation was performed in anesthetized dogs with renal hypertension (experimental perinephritis) before and thirty minutes after intravenous hexamethonium bromide one mg./kg. Average mean arterial blood pressure promptly decreased from 178 to 83 mm. Hg. and stabilized at 119 mm. Hg. during the second flow determination. Blood pressure reduction was accompanied by a sinus tachycardia. Cardiac work was decreased due to the reduction in mean arterial blood pressure and cardiac output. Coronary blood flow increased slightly, and coronary vascular resistance was slightly reduced during blood pressure reduction.

Hexamethonium bromide administered parenterally resulted in a significant reduction in mean arterial blood pressure in human subjects with sustained essential hypertension and in renal hypertensive dogs. The cerebral and coronary volume flow was maintained during the period of blood pressure reduction under the conditions described.

Hemodynamic and Renal Changes after Pericardiectomy in a Patient with Calcified Constrictive Pericarditis. JAMES W. CULBERTSON, WILLIAM H. AMES, WALTER M. KIRKENDALL, JOHANN L. EHRENHAFT, ERNEST O. THEILEN and STEVEN M. HORVATH, Iowa City, Iowa. (Introduced by Horace M. Korns).

An unusual experience with repeated physiological evaluation of dramatic results of pericardiectomy for *concretio cordis* of unknown etiology in a girl of 16 years is reported. Hospitalization followed progressive disability related to exertional dyspnea and peripheral edema (weight gain from 130 to 156 pounds in 6 months). Vital capacity was 3100 ml, venous pressure 230 mm saline, and Decholin circulation time 28 seconds. Electrocardiogram showed low amplitude QRS complexes. Roentgenkymogram demonstrated practically absent ventricular contraction amplitude. Cardiac catheterization revealed elevated right atrial and pulmonary arterial pressures (32/24 and 55/45 mm Hg, respectively) and low normal resting cardiac output (5.0 L/min.). Femoral intra-arterial pressure was 105/60. Glomerular filtration rate (102 ml/min.) and Tm-PAH (106 mg/min.) were normal, but renal plasma flow was strikingly reduced (288 ml/min.) and filtration fraction correspondingly elevated (0.35). Adequate therapeutic trial of bed rest, dietary sodium restriction, and Mercuhydrin failed to provoke diuresis.

Spontaneous diuresis occurred promptly after complete pericardiectomy. Within two weeks the patient's weight had fallen sharply to 135, venous pressure to 140, circulation time to 13, and right atrial and pulmonary arterial pressures to 8/0 and 28/9, respectively. Increases had occurred in amplitude of QRS complexes and ventricular border movement, resting cardiac output (+20%), and femoral arterial pressure (to 125/75). Renal clearance data had changed strikingly to near normal values: GFR 132, RPF 531, FF 0.25, and Tm-PAH 105.

Subsequently the patient has been asymptomatic and working 10 hours daily as a waitress. Comprehensive studies were repeated 4 and 12 months postoperatively and no evidence of impaired cardiac, respiratory, or renal function found. Cardiac output now increases normally on demand, largely through increase in stroke volume. Review of the hemodynamic changes raises the question whether chronic systemic venous distension may not have been of considerable importance in causing the preoperative renal dysfunction.

Comparison of Some Methods for Studying Fat Absorption Illustrated in a Case of Pancreatic Insufficiency. PERRY J. CULVER, W. WARREN POINT and MILTON C. HOFFMAN, Boston, Massachusetts. (Introduced by Chester M. Jones).

To date the study of fat absorption in man has lacked adequate methods to show both rate and quantity of fat absorption. Gravimetric balance studies are tedious and yield only quantitative information. Blood fat absorption curves shown by lipid carbon determinations, lipo-krit measurements and chylomicron counts are poor approximations of the rate of fat absorption, since each determination represents the component of absorption, mobilization, utilization and deposition of fat.

In the hope of providing a method to measure both quantity and rate, absorption of olive oil labelled with radioactive iodine was compared with the other methods in a patient with pancreatectomy for pancreatic carcinoma. On a constant daily dietary intake of 180 grams of fat, there was a fecal fat loss of 78 per cent of intake. Remarkably similar was the recovery in the stools of 78 per cent of administered radioactivity following a test meal of 30 grams of radioactive iodine labelled olive oil. Blood fat absorption curves after this same test meal were flat

by chylomicron, lipokrit and lipid carbon measurements. A definite rate of fat absorption, however, was shown by the rise in serum radioactivity and recovery of 21 per cent of the administered radioactivity in the urine. By modifying the control conditions, with resultant improvement in fat absorption, the gravimetric method of balance studies correlated well with the radioactive isotope recovery in the stool.

Close correlation between fecal fat losses measured gravimetrically and isotopically plus parallel changes in blood fat absorption curves under the various conditions of this experiment suggest that olive oil labelled with radioactive iodine is a rapid and accurate method for determining both quantity and rate of fat absorption.

Studies of Absorption from the Human Small Intestine by an Intubation Technique: The Effect of Variations in Blood Sugar on the Rate of Absorption of Glucose. ALVIN J. CUMMINS, New York, N. Y. (Introduced by Thomas P. Almy).

The rate of absorption of solutions of glucose (5% and 10%) and methionine (0.25% and 0.50%) from the upper small intestine was measured in 17 subjects, using a modified Nicholson-Chornock multiple lumen tube. These solutions were delivered into the intestine at constant rates, and the unabsorbed residue was aspirated 45 cm. below the point of inflow.

The absolute rates of absorption for glucose and methionine, as determined by this method, varied widely from person to person. In 7 subjects, the greatest variation in absorption rate in two identical and consecutively performed tests was 25% for glucose and 50% for methionine.

Cori found in experiments on rats that the absorption of 25–80% glucose was independent of the concentration fed. Within the more physiologic range of concentrations studied by us, the rate of absorption of both glucose and methionine was significantly greater from the more concentrated solutions. This indicates that the human small intestine may not conform to "Cori's law" of glucose absorption.

Production of hyperglycemia by intravenous glucose or of hypoglycemia by intravenous insulin failed to alter significantly the rates of absorption for glucose and methionine.

Observations on the Use of Protoveratrine in Hypertensive Vascular Disease. J. H. CURRENS, G. S. MYERS and J. S. MCGINTY, Boston, Mass. (Introduced by E. F. Bland).

Twenty-four patients have been observed from six to thirty months (average thirteen) while receiving protoveratrine in an effort to control hypertension. Ten patients described hypertension headaches and in nine the relief was classified as good. Five patients had symptomatic hypertensive heart disease all of whom had significant improvement.

Seven patients with toxemia of pregnancy have received protoveratrine for three to eight weeks with apparent beneficial effect on blood pressure and course of pregnancy.

Protoveratrine was administered orally usually after meals and bedtime, in dosage ranging from 0.4 mg. to 1.5 mg. Enteric coated protoveratrine at bedtime was useful to control early morning hypertension. Adverse symptoms such as nausea, vomiting, salivation, giddiness, and weakness were noted by all patients at some time during treatment one half to two hours after medication. Delicate regulation of individual dosage by 0.05 mg. usually adjusted by patient, reduced these adverse reactions to a minimum and infrequently precluded continuous administration. The patients noticed transient hemiparesis following emesis at time of maximal sedative effect. No significant sequelae have been observed after toxic reactions. One patient received 50 mgs. without disaster.

Renal clearance of para-amino hippuric acid after intravenous protoveratrine was observed to decrease in the first thirty minutes associated with abrupt fall of blood pressure. Clearance values usually returned to control levels during two subsequent thirty minute periods with blood pressure still reduced.

Cardiac catheterization has revealed significant fall in cardiac output in most patients after intravenous protoveratrine. Decrease in mean blood pressure and stroke output after protoveratrine results in appreciable fall in left ventricular work index. Elevated pulmonary arterial pressure decreased to normal after protoveratrine. Atropinization in two patients failed to prevent significant slowing of heart rate after protoveratrine.

Protoveratrine administration is feasible as long term treatment of hypertensive vascular disease in some patients.

The Influence of the Tonicity of the Body Fluids on the Rate of Excretion of Electrolytes. J. H. DARRAGH, L. G. WELT, A. V. N. GOODYER and W. A. ABELE, New Haven, Conn. (Introduced by David M. Kydd).

The influence of the tonicity of the body fluids on the rates of excretion of sodium, chloride, potassium, and ammonia was examined in four healthy young adult male subjects.

An infusion of an isotonic or hypertonic solution of sodium chloride was administered after a control period during which the tonicity of the body fluids was either: 1) not altered, or 2) reduced by achieving and maintaining a positive balance of water of about two liters. This latter was accomplished with infusion of a solution of five per cent glucose in water. Pitressin was included in the glucose infusion to reduce urine volume to a minimum and thus to facilitate the maintenance of the positive balance of water. Each of the four subjects served as his own control and was studied with and without dilution of the body fluids. Similar amounts of pitressin were given in the control studies.

The rates of excretion of sodium and potassium were lower in the dilution studies than in the control experiments where the tonicity of the body fluids had not been decreased. In contrast, the rate of excretion of ammonia was higher in the dilution studies.

The slower rate of excretion of sodium in the dilution studies was influenced, presumably, by the decrease in the

effective osmotic pressure of the extracellular fluid or its necessary consequence—an increase in cellular hydration. Since cellular dehydration is alleged to augment the excretion of potassium, it may be that the diminished rate of excretion of this ion in the dilution studies is due to cellular overhydration.

The increase in the rate of excretion of ammonia may have been the consequence of the demand for the continued excretion of cation at a time when there was a diminished rate of excretion of sodium and potassium.

A Disturbance in Inositol Metabolism in Diabetes Mellitus. W. H. DAUGHADAY,* J. LARNER and ELLABETH HOUGHTON, St. Louis, Missouri.

Although increased excretion of inositol in diabetes mellitus has been reported several times since 1864, this observation has been generally overlooked or attributed to polyuria. Now that inositol has been found to be a structural component of cephalin and probably is concerned with hepatic lipid metabolism, its increased excretion in diabetes mellitus merits reinvestigation.

The daily inositol excretion of five normal subjects on an unrestricted diet has been measured microbiologically using S. Carlsbergensis. The average inositol content of 15 urine specimens examined was 56 mg per day (range 26 to 144 mg per day). Two normal subjects excreted an average of 30 mg per day of inositol with an average urine volume of 907 ml. When the urine volume was increased to 2782 ml, the inositol excretion rose to only 46 mg per day.

Repeated observations were made of the excretion of inositol by six hospitalized patients with uncontrolled diabetes mellitus. In every case the inositol excretion was high. The maximum excretion by each patient was 280, 341, 350, 518, 581 and 851 mg per day. The amount of inositol found in the urine correlated well with the degree of glycosuria. When glycosuria was adequately controlled by insulin treatment the excretion of inositol fell toward normal. In both the normal and diabetic subjects the urinary inositol appeared to be in the free form.

Similar changes in inositol excretion have been observed in partially depancreatized rats. Inositol excretion in these animals is largely dependent on dietary inositol. At any given oral intake the diabetic rats excreted much more inositol than did the normal animals.

It is concluded that there is a defect in inositol metabolism in diabetes mellitus which cannot be explained by differences in inositol intake or urine volume.

Separation of Primary Amines from Urine and Blood. DEAN F. DAVIES and KATHRYN M. WOLF, St. Louis, Missouri. (Introduced by John R. Smith).

Methods developed in this laboratory for the demonstration and separation of primary amines have been used in the study of their presence in normal, pooled urine, and in 24 hour collections of normal and hypertensive urine. Samples of pooled male urine collected under toluene were brought to pH 10 and centrifuged. Continuous extraction of the supernatant was carried on for two four-hour periods, the urine hydrolyzed in acid, made alkaline again, and re-extracted four hours. Concentrates of the extracts were chromatographed by the ascending technique and sprayed with specially prepared ninhydrin solution. Six hitherto unrecognized amine substances were identified and, by previous experience with known amines, their nature could be inferred. The heaviest and most consistent appears to be a 4–6 carbon aliphatic primary amine having a prolonged pressor action. Its Rf is close to that of isoamylamine (0.5), but evidence against their identity include electron diffraction patterns and characteristics of color development. Other urinary amines appear either to be of shorter chain length or to contain hydrophilic groups.

Attempts to correlate the appearance of these compounds with the hypertensive state in twenty-two 24-hour samples from six subjects indicate no distinct difference between normotensives and individuals with benign hypertension. However, malignant hypertensives have altered patterns. One patient with the endocrine hypertensive syndrome had large quantities of a histamine-like amine. Administration of organic nitrates when compared with control periods on placebo appears to decrease the output of amines in non-endocrine individuals.

In blood, several amines appear and two are interpreted to have longer aliphatic moieties than isoamylamine. One may be a small volatile pressor substance previously found.

Evidence for an Altered Enzyme Resulting from Mutations to Drug Resistance or Nutritional Requirement. BERNARD D. DAVIS * and WERNER K. MAAS, New York, N. Y.

Conceivable biochemical mechanisms of drug resistance include: (1,2) decreased penetration or increased destruction of the drug; (3) increased synthesis of an antagonistic metabolite; (4) increased concentration of the enzyme utilizing this metabolite; (5) more efficient utilization of the product of this enzyme; (6) an alternative metabolic pathway; and (7) formation of an enzyme with decreased affinity for the drug or increased affinity for the metabolite.

Discrimination among these mechanisms became possible with the discovery that *p*-nitrobenzoic acid (PNB) inhibits *Escherichia coli* by two independent mechanisms, competing simultaneously with *p*-aminobenzoic acid (PAB) and *p*-hydroxybenzoic acid. In the presence of PNB plus either metabolite, mutants resistant to PNB competition with the other metabolite could be selected. Since these two types of mutants were found not to be cross-resistant, mechanism 1 and 2 are excluded for these strains. Furthermore, resistance to PNB competition with PAB was found to differ from resistance to sulfonamide competition with PAB. This finding excludes mechanisms 3 to 6, which could not discriminate between different analogues. These results therefore point to either mechanism 7 or some unknown mechanism.

Further, more direct support for an altered enzyme was next provided by the observation that increased resistance to a sulfonamide is associated with a proportional increase

in the sulfonamide/PAB competitive ratio. Finally, while a rigorous proof of enzyme alteration was not possible for drug resistance, since the PAB-utilizing enzyme could not be studied in extracts, conclusive proof for another kind of qualitative alteration has been achieved with another enzyme: the pantothenate-synthesizing enzyme extracted from a mutant which requires pantothenate only at temperatures above 30° differs from the corresponding wild-type enzyme in being extraordinarily sensitive to heat denaturation.

This ability of mutations to lead to qualitative as well as apparently quantitative changes in enzyme production is significant for other medical problems besides drug resistance, such as hereditary diseases, neoplastic growth, and variations in microbial virulence.

The Acceleration of Clotting by Platelets Treated with Thrombin Preparations. JANE F. DESFORGES and FREDERICK S. BIGELOW, Boston, Mass. (Introduced by Clark W. Heath).

Platelets plus a plasma factor are known to produce a thromboplastic effect in blood coagulation. To determine whether there is enhanced platelet activity once thrombin is formed, the effect of platelets treated with various thrombin preparations was observed in several phases of clotting.

Platelets washed in saline were incubated at room temperature with thrombin preparations and then washed to remove free thrombin. These platelets were found to shorten whole blood clotting and recalcification times to a degree not observed with washed untreated platelets.

The effect of treated platelets on prothrombin consumption and on two-stage prothrombin tests demonstrated increased prothrombin consumption and acceleration of thrombin formation in normal and in hemophiliac plasma. This action was evident in the absence of added thromboplastin but was not demonstrable either with thrombin preparations or with untreated platelets alone.

Thus, washed platelets exposed to thrombin possess thromboplastic activity not noted with untreated platelets. This action of treated platelets suggests a further means by which coagulation proceeds at an increasing rate.

The treated platelets showed no consistent change in stickiness, agglutination, electrophoretic mobility or microscopic appearance.

Urinary Alpha-Ketolic Corticoids Determined by Paper Chromatography in Patients with Hypo- and Hyperfunction of the Adrenal Cortex. F. C. DOHAN * and E. M. RICHARDSON, Philadelphia, Pennsylvania.

The alpha-ketol group is characteristic of metabolically active corticoids and some of their metabolites. This study has demonstrated the expected marked decrease in urinary excretion of the alpha-ketolic steroids in patients with Addison's or Simmond's Disease and the marked increase in Cushing's Syndrome. The pattern of excretion in Cushing's Syndrome is similar to that produced by intravenous injection of ACTH. The urinary steroids were hydrolyzed by glucuronidase followed by acid hydrolysis during chloroform extraction. Paper chromatography was performed by a modification of the methods of Zaffaroni, Burton, and Keutmann and of Tennent, Whitla, and Florey. This permits the use of a small aliquot of the 24 hour urine collection for the detection (with blue tetrazolium) and semi-quantitative measurement of steroids containing the alpha-ketol group. Identification has, in general, followed the technics of Zaffaroni et al.

Following the injection of 10–20 mg. of ACTH intravenously in two hypertensive men, at least 9 alpha-ketols have been detected in the 3 chromatographic fractions. In the most polar fraction, tetrahydrocortisone and a closely associated, highly polar compound (probably tetrahydro-hydrocortisone), reduction products of compounds "E" and "F", are excreted in large amounts in normal individuals, and found increased in Cushing's Syndrome, and greatly increased after ACTH. Hydrocortisone ("F") was found in markedly increased amounts in Cushing's Syndrome and after ACTH injection. At least two alpha-ketols are found in the cortison ("E") region after ACTH and possibly in Cushing's Syndrome. Further identification studies are in progress.

After ACTH, and in Cushing's Syndrome, 3 alpha-ketols have been detected in the second fraction, and one in the third, least polar fraction. Those in Cushing's Syndrome appear to be similar to those found after ACTH administration. Little or none of these substances was found in the urine of untreated patients with Addison's or Simmond's Disease.

Microscopic Correlation of Vascularity with Glandular Secretion in the Stomach of Man in Vivo. RONALD K. DOIG and STEWART WOLF,* New York, New York.

In the fistulous subject, Tom, it has been possible with a dissecting microscope to examine and photograph the venous channels which surround the necks of the simple branched tubular glands in the body of the stomach. Simultaneous measurement of the production of acid, pepsin and glandular mucoprotein showed a direct correlation between vascularity and glandular function. The variation in size of the vascular channels between the state of hypersecretion and of glandular hypofunction with anacidity was greater than 100 per cent. It is of incidental interest but of unexplained significance that the membrane fluoresces under filtered ultraviolet light in the presence of active glandular function and that the extent and intensity of the fluorescence is roughly proportional to glandular function. Colored drawings of the fluorescence will be shown as well as photographs in color of the microscopic appearance of the gastric mucosa for the first time in a human being in vivo.

Prevention of Experimental Arthritis by Combined Treatment with Phenergan and Irgapyrine. PIERRE DUCOMMUM, Montreal, Canada. (Introduced by Hans Selye).

On the basis of previous experiments concerned with the anti-inflammatory potency of some anti-pyretics and anti-histaminics, we have studied the anti-phlogistic properties of the association of Phenergan (dimethyl-amino-

2-methyl-1 ethyl N di-benzoparathiazine) and Irgapyrine (dipyrine and 3,5,-dioxo-1,2 diphenyl-4-n-butyl-pyrazolidin-Natrium) upon experimental arthritis in intact and adrenalectomized rats.

The development of the tropical arthritis was prevented under the influence of this combined treatment with Phenergan (6 mg) and Irgapyrine (30 mg), subcutaneously injected once daily. The prevention observed was almost as complete as that obtained with cortisone treatment (2.5 mg daily).

Further investigations showed that this anti-arthritic action could be produced in adrenalectomized rats, thus indicating that hypophyseal adrenal stimulation was not responsible for the anti-phlogistic effect.

Other combinations of such related compounds were investigated, but Phenergan-Irgapyrine was found to be the most potent association.

The parallelism between the action of cortisone and Phenergan-Irgapyrine is also found on the connective tissue permeability level: Phenergan-Irgapyrine, like cortisone, decreases the spreading of subcutaneously injected hemoglobin in intact and adrenalectomized rats.

Under the influence of this combined treatment, the hepatic glycogen content in fasting adrenalectomized rats remains unchanged, while the anti-arthritic effect is prominent.

We report these results merely to stimulate interest in the possible clinical applications of such drug combinations.

Endogenous Fat Metabolism Studied by Heavy Water Dilution and Nitrogen Balance. I. S. EDELMAN, L. BROOKS, and F. D. MOORE,* Boston, Mass.

Previous studies from this laboratory have established the feasibility of measuring total body water in the normal living subject by deuterium oxide dilution. We have now extended our work on body water to patients suffering from a variety of illnesses. We find that this method, when combined with nitrogen balance, yields information on fat metabolism. This forms the subject of the present communication.

The human body is composed of fat, lean tissue solids and water. The mineral fraction of the body is about 7% by weight, largely situated in the skeleton. Carbohydrate totals less than 1% of body weight. Estimation of *in vivo* changes in body fat content is therefore made possible by direct measurement of the nitrogen balance and total body water, and calculation of the fat changes by the following equations.

(1) Body weight = fat + lean tissue solids + water.
(2) Δ lean tissue solids = Δ nitrogen balance $\times$ 8.1.
(3) Δ body fat = Δ body weight—(Δ water + Δ lean tissue solids).

Alternative expressions for the interrelationships of fat, water and lean tissue have evolved from the work of Pace and Behnke on body fat content and specific gravity. These expressions are valid in the absence of changes in hydration. The above equations are generally valid, however, even where acute disease is present and are independent of changes in lean tissue hydration.

A series of studies will be reported in which changes in body water, lean tissue solids and body fat were measured by these methods. Utilization of fat at rates up to 170 grams per day (a caloric equivalent of 1530 calories per day) is not uncommon in acutely ill patients. Fat redeposition in convalescence usually occurs at a rate in the vicinity of 50 to 75 grams per day.

Composition and Distribution of Plasma Lipoproteins in Normal and Pathological States. HOWARD A. EDER and ELLA M. RUSS, New York, N. Y. (Introduced by David P. Barr).

Recent studies have shown that essentially all the cholesterol and phospholipid of the plasma are combined with protein either as alpha or beta lipoproteins. When these lipoproteins are separated by Cohn fractionation, their composition can be studied and the distribution of the plasma lipids between the two groups determined. In atherosclerosis as well as in nephrosis and diabetes, the distribution of cholesterol has been found to be abnormal. In the present study the distribution of the other plasma lipids has been determined in order to understand better the role of lipoproteins in the pathogenesis of these diseases.

In normal fasting individuals about one third of the plasma neutral fat was found in alpha lipoprotein, and the remainder was in beta. When plasma concentration of neutral fat was increased either by ingestion of neutral fat or in disease such as nephrosis, the increment was found almost entirely in beta lipoprotein. Following the administration of heparin, there was no change in the total plasma lipids or in the distribution of cholesterol or phospholipid. The effect of heparin on visible lipemia must therefore be considered in terms of its effect on neutral fat distribution.

In patients with biliary cirrhosis and in the early stages of acute hepatitis, the lipoproteins are qualitatively different from those found in normals and in all other diseases thus far studied. In beta lipoprotein the ratio of cholesterol to phospholipid was less than half that usually found and approached the ratio found in alpha lipoprotein. Normally the ratio of non-esterified to total cholesterol in the lipoproteins varies only slightly from that of whole plasma. In these patients the cholesterol of alpha lipoprotein may all be free cholesterol with beta lipoprotein containing all the esterified and some free cholesterol. With recovery from hepatitis the lipoproteins return to the normal pattern.

Hemodynamic Studies in a Case of Tricuspid Stenosis. M. IRENE FERRER,* RÉJANE M. HARVEY and ANDRÉ COURNAND, New York, N. Y.

The hemodynamics of the circulation in a patient with sinus rhythm and tricuspid stenosis of rheumatic origin, proven later by necropsy, were studied using the cardiac catheterization technique. The pressure curves in the right auricle and ventricle—the compartments on either

side of the stenosis—provide the background for a discussion of the detailed performance of these chambers. As might be expected, the pressure in the right auricle remained higher than in the right ventricle throughout diastole. Three other unusual discrepancies were noted: (a) the end diastolic point in the right auricle and ventricle were not identical, as occurs in normal or even dilated hearts, and (b) "isometric" contraction of the right ventricle was in progress for a longer than normal time before the tricuspid valve closed, and finally (c) there was no visible "c" wave in the auricular tracing, eliminating the possibility of significant tricuspid regurgitation and indicating that there was probably downward fixation of the leaflets preventing an upward systolic bulge. Other features of the curves indicated continuous though varying atrioventricular flow throughout diastole and absence of early rapid diastolic ventricular filling. The response to exercise in this patient, who also had mitral stenosis, suggested that the lack of increase in cardiac output during effort may have been due to the tricuspid stenosis itself which impounded the increasing venous return in the auricle and largely prevented this increase from reaching the ventricle or pulmonary bed. Hence the rise in pulmonary artery pressures during exercise in this woman could be ascribed only to the effects of increased heart rate in face of a mitral stenosis.

Since autopsy findings permitted the measurement of the area of the stenosed tricuspid valve, the hydraulic formula for estimation of valve area was applied using the hemodynamic data. The observed and calculated valves were not identical.

The Significance of Plasma Iron Turnover. STUART C. FINCH and JOSEPH F. ROSS,* Boston, Massachusetts.

The plasma iron has physiological significance, since it is iron in transport and is in dynamic equilibrium with other fractions of body iron. Variations in plasma iron concentration reflect physiological and pathological variations in iron metabolism and hematopoiesis. The rate of turnover of plasma iron is of greater potential interest than is the plasma concentration, since turnover rates may be expected to provide fundamental information relative to the kinetics of iron metabolism and hematopoiesis, and may have definite clinical diagnostic value.

Employing radioiron as a biologic tracer, we have investigated the kinetics and clinical significance of plasma iron turnover in human subjects and in dogs. The influence on plasma iron turnover of alterations in rates of erythrocyte production, in plasma iron levels, in size of total body iron stores, and in the functional capacity of the reticuloendothelial system has been evaluated.

Our studies indicate that the amount of plasma iron turnover is primarily a reflection of the amount of red blood cell formation. The rate of plasma iron clearance also is primarily a reflection of the red blood cell formation and is only secondarily affected by reticuloendothelial function and plasma iron level. Other factors exert a definite but small influence on plasma iron turnover. The rate of erythrocyte formation is reliably indicated by this amount of plasma iron turnover and this determination provides a rapid and useful method of assessing erythropoietic rates. Finally, it becomes apparent that no reliance can be ascribed to oral plasma iron tolerance tests as an index of iron absorption, unless the plasma volume and iron clearance rates are known.

Failure of Equilibration of Inulin in Anuric Subjects. JOHN T. FINKENSTAEDT, MAURICE P. O'MEARA and JOHN P. MERRILL, Boston, Massachusetts. (Introduced by James P. O'Hare).

Measurement of the volume of distribution of inulin following a single injection was attempted in 14 anuric patients and 4 nephrectomized dogs. Failure to equilibrate was observed since serum inulin levels fell continuously. This could not be explained by urinary or other losses, or by interfering substances in the sera. All subjects had restricted fluid intake and lost weight.

In 12 patients given 4.5 to 13 gm. of inulin, the apparent "inulin space" at 8 hours averaged 24% (20.8–31.0%) of body weight. The 24 hour inulin space in 10 patients averaged 30.2% (23.5–37%). Spaces of 4 patients followed for 96 hours averaged 45.2% (31.7–53.6%). In 2 of these patients, 8 hour spaces determined at 48 hours, after a second injection of inulin, were similar to those after the initial dose. Preinjection inulin blanks ranged from 2.8–9.5 mgm.%. Urinary recoveries during diuresis were inaccurate because of high urinary blanks.

In 3 bilaterally nephrectomized dogs given large doses of inulin (1.0 gm/Kg.) inulin concentrations fell continuously over 5–7 days of survival without exogenous loss. The rate of disappearance of inulin averaged 0.5% of the administered dose per hour. By 150 hours, the apparent "inulin spaces" were 50% of body weight. In 1 dog given a dose more comparable to the patient dose (0.2 gm/Kg.), the serum level approached the blank value in 120 hours.

It is concluded that inulin cannot be used to measure "extracellular space" in anuric subjects because of its failure to equilibrate. Whether this is the result of catabolism, transfer into cells, entry into the reticuloendothelial system or other compartments is unknown.

The Effect of Corticotropin Administration on Blood Bile Acids and Pruritus in Biliary Cirrhosis. EDMUND B. FLINK and CHARLES F. WILLIAMS, Minneapolis, Minnesota. (Introduced by F. W. Hoffbauer).

Corticotropin was administered to four patients with primary biliary or cholangiolitic cirrhosis and one patient with secondary biliary cirrhosis. The most significant alterations were a decrease in blood bile acids and a decrease in pruritus.

Before corticotropin administration the blood bile acid concentrations were elevated to two to three times the upper limit of normal value (1.0 mg./100 cc.). During treatment of the first patient the bile acid level fell in 12 days from 2.0 mg./100 cc. to 0.8 mg./100 cc. and pruritus disappeared. One month later the pruritus returned to its previous severity and the bile acid concen-

tration had risen to 2.3 mg./100 cc. Therapy for 7 days caused a drop in bile acids to 1.2 mg./100 cc. and nearly complete alleviation of the pruritus. The second patient experienced only slight diminution in pruritus, and the bile acid level decreased from 2.7 mg./100 cc. to 2.0 mg./100 cc. in 12 days. The third patient had only mild pruritus initially, but it diminished during therapy. The bile acid level decreased from 3.0 mg./100 cc. to 2.0 mg./100 cc. in 8 days. The fourth patient noted nearly complete relief from pruritus and the bile acids decreased from 3.6 to 2.1 mg./100 cc. The fifth patient (secondary biliary cirrhosis) noted complete relief from pruritus, and the bile acids decreased from 3.2 mg./100 cc. to 1.6 mg./100 cc.

The decrease or disappearance of pruritus in these patients at a time when bile acid concentrations were decreasing rapidly and a return of pruritus when bile acid levels rose again are in accord with the concept that bile acids have some relationship to pruritus. The decrease in bile acids cannot be accounted for by a decrease in the degree of regurgitation jaundice. It is possible that bile acids were used in the synthesis of adrenal corticoids or that an increased biliary excretion was induced.

The Effect of the Continuous Removal of Measured Amounts of Spinal Fluid on Cerebro-Spinal Fluid Dynamics. F. F. FOLDES, E. KEUTMANN and R. D. HUNT, Pittsburgh, Pennsylvania. (Introduced by P. C. Zamecnik).

0.2 cc., 0.4 cc., 0.6 cc., and 0.8 cc. of spinal fluid per minute were removed from four patients each over a 60 minute period. In another four patients 3 cc. per minute of spinal fluid were removed over a 20 minute period. The cerebro-spinal fluid pressure, pulse rate, and blood pressure were observed every 5 minutes. At the end of the experiments the original cerebro-spinal fluid pressure was re-established by the subarachnoidal injection of normal saline. When 0.2 to 0.4 cc. of spinal fluid per minute were removed, after an initial decrease, the level of the spinal fluid pressure became stabilized. When larger quantities of spinal fluid were removed, there was a progressive decline in the spinal fluid pressure all through the experiment. When 3 cc. of spinal fluid per minute were removed, the spinal fluid pressure dropped from an average of 200 mm. to 10 mm. of water in 20 minutes. It was found that consistently less saline was necessary to re-establish the original spinal fluid level than the amount of spinal fluid removed in the course of the experiment. The relationship between the amount of spinal fluid removed and the amount of saline necessary for the re-establishment of the original spinal fluid level provided interesting data on some aspects of the mechanism of spinal fluid secretion.

Ventilatory Function in Mitral Stenosis. ROBERT N. FRANK, DAVID W. CUGELL and EDWARD A. GAENSLER, Boston, Mass. (Introduced by Laurence B. Ellis).

The ventilatory effects of pulmonary vascular changes are well demonstrated in mitral stenosis. This disease usually evolves in two distinct phases: Initial pulmonary hypertension without increment in blood volume, and terminal congestive circulatory failure with increase in blood volume.

Sixty-two patients were studied in whom mitral stenosis was the predominant valvular lesion. They were classified according to the New York Heart Association criteria of functional limitation. Patients with complicating pulmonary disease were excluded.

In patients with asymptomatic mitral stenosis, all measurements of pulmonary function during rest and exercise were normal. With increasing disability there was progressive reduction of maximum breathing capacity, total and timed vital capacity. In severe disability, the total lung capacity was decreased chiefly at the expense of the vital capacity. The associated relative increase in residual volume was not attended by a defect in intrapulmonary mixing. Patients with slight limitation of activity experienced discomfort during walking exercise. However, they did not hyperventilate. The breathing reserve was not significantly depleted until disability was marked.

By comparison, patients with pulmonary disease with equivalent degrees of incapacity showed a greater ventilatory defect. In restrictive insufficiency the maximal breathing capacity, vital capacity, and total lung capacity showed greater reduction than in mitral stenosis. In obstructive insufficiency, the maximal breathing capacity was less, the total lung capacity and residual volume greater, and the intrapulmonary mixing index abnormally elevated. The breathing reserve in pulmonary disease was characteristically smaller. The patients with mitral stenosis, unlike those with pulmonary disease, were disabled disproportionately to the measured defects in pulmonary function.

The results were ascribed to two separate mechanisms. Initially, pulmonary hypertension reduced lung compliance and interfered with maximal flow velocity. With the advent of circulatory failure and blood volume increase, the total lung capacity was reduced.

The Thyroid Hormonal Pool in Man and Dog. A. STONE FREEDBERG,* LEONARD WOLSKY, GEORGE S. KURLAND and MILTON W. HAMOLSKY, Boston, Massachusetts.

Radioactive iodine studies have made significant contributions to our understanding of many aspects of thyroid function. Available methods, however, have not yielded conclusive information concerning certain basic problems. The infusion of labelled thyroid hormone was utilized to study two such problems: (1) The size and turnover of the hormonal pool in normal and abnormal states of thyroid function and (2) the nature of the hormone elaborated by the thyroid gland in various metabolic states. The thyroid hormonal pool in man and dog was studied by determining the rate of disappearance of protein-bound I^{131} ("radioiodothyroglobulin") obtained from humans treated by I^{131}. Following intravenous infusion of such plasma, recipient's blood was analyzed frequently over 23–70 hours for remaining total and protein-bound I^{131}. In each experiment there was a rapid

initial exponential decrease and a slower terminal exponential decrease. Standard Graphic analysis revealed: I: In human recipients, the average value for the total exchangeable "thyroglobulin" (μgms) in four euthyroid subjects was 399 ± 44 vs. 1142 in a thyrotoxic and 65.0 in a patient with myxedema. Circulating "thyroglobulin" (μgms) averaged 173.5 ± 23.8 in 4 euthyroids vs. 557 in the thyrotoxic and 23.0 in the myxedemic patient. The turnover of total "thyroglobulin" (μgms/day) averaged 119.4 ± 11.4 in the euthyroids vs. 480 for the thyrotoxic and 26.2 for the myxedemic patient. II. In dog recipients, following infusion of such tagged plasma from 2 euthyroids the turnover time was 37.9 and 30.2 hours, the half-time was 26.3 and 21 hours and the turnover rate, 63.2 and 79.2 %/day. Infusion of plasma from 2 thyrotoxics yielded turnover times of 18.1 and 17.8 hours, half-times of 12.6 and 12.4 hours and turnover rates of 132.0 and 134.0 %/day. These observations demonstrate a clear difference in the size of the exchangeable thyroid hormonal pool, as well as in the daily turnover, in various states of thyroid function. They also denote a basic difference in the nature of the thyroid hormone elaborated by patients with thyrotoxicosis as contrasted with euthyroid individuals.

A New Method for Measuring Transcapillary Exchanges: The Transfer of Salt and Water in the Lesser Circulation of Man. NORBERT FREINKEL, GEORGE E. SCHREINER and JOHN W. ATHENS, Washington, D. C. (Introduced by Robert W. Berliner).

The Stewart Principle has been modified for the evaluation of net exchange of micromolecular solutes across capillary beds. Flow measurements based upon the mean dilution of an indicator during its first circulation will be artifactually elevated if a portion of the indicator is diverted from the stream during that circulation. Hence, if a crystalloid is administered simultaneously with a colloid, differences in blood flow determined separately from their concurrent dilutions must reflect the net extravascular (i.e. transcapillary) loss of the more diffusible agent.

This technique of simultaneous indicators was applied to a study of net salt and water transfers in the lesser circulation of man. T1824 was employed as the intravascular standard. Such use of T1824 was validated by the demonstration of instantaneous and complete dye-protein union during the first circulation.

T1824 in various combinations with I^{131}-labelled albumin, inorganic I^{131}, Na^{24} and D_2O was administered to 24 subjects. Delivery was made through a catheter within the subclavian vein. Flow values were calculated from the extrapolated semilogarithmic plot of arterial concentrations during the first circulation. The blood flow ratio T1824 Indicator/Crystalloid Indicator was used as an index of relative "virtual" distribution compartments. A ratio of unity denotes no net transfer. After correction for red cell penetration, T1824/Inorganic I^{131} flow ratios averaged 1.015 (± 0.03) and T1824/Na^{24} ratios averaged 0.997 (± 0.04). This method did not disclose any significant transcapillary exchange or extravascular dilution of electrolytes within the lesser circulation of normal man.

The T1824/Na^{24} flow ratio in a patient with mitral stenosis and proven pulmonary hypertension was 0.902. In another subject with a large carotid arteriovenous fistula, the T1824/Inorganic ratio was 0.881. Both values are suggestive of transcapillary electrolyte exchange. Potentialities of the simultaneous indicator technique in abnormal states will be discussed.

The Hemodynamic Effects of Hexamethonium in Man. EDWARD D. FREIS, JOHN C. ROSE, THOMAS F. HIGGINS, ROBERTS T. KELLEY, HAROLD W. SCHNAPER and ROBERT L. JOHNSON, Washington, D. C. (Introduced by Harold Jeghers).

The reduction of arterial pressure following the intravenous administration of hexamethonium in supine subjects is accompanied by no or a slight reduction of cardiac output (Fick) and by a significant decrease in total peripheral resistance. The changes in local vascular resistances vary markedly with the vascular area under study. Thus, muscle blood flow (calf segment) usually decreases, whereas foot and digit flows increase many fold. Effective hepatic-portal blood flow usually does not change significantly indicating a decrease in peripheral resistance, while renal blood flow decreases initially and then slowly returns toward control levels. In the early hypotensive period there is a decrease in renal plasma flow, glomerular filtration rate and filtration fraction accompanied by moderate oliguria. The oliguria is not as marked nor the return of clearance values to the baseline as rapid as after veratrum viride.

These studies suggest that the autonomic blockade following hexamethonium does not produce a uniform decrease in peripheral vascular resistance. In the presence of a generalized inhibition of the sympathetic nervous system the percentage changes in peripheral resistance vary greatly in different vascular areas. It is suggested on the basis of this and other evidence that the sympathetic vasoconstrictor nerves are of great importance in maintaining vascular tone in certain regions such as the hand and feet and of much less importance in other vascular areas such as the kidneys and muscles.

The Cholate Mechanism and Its Role in the Production of Hypercholesteremia Found in Clinical and Experimental Nephrosis. MEYER FRIEDMAN,* SANFORD O. BYERS and RAY H. ROSENMAN, San Francisco, California.

Nephrotic patients having hypercholesteremia, similar to other types of hypercholesteremic patients, were found to have an increase in their plasma cholate content. Thus, in fifteen nephrotic patients, the average plasma cholesterol was 695 mg./100 cc., and the plasma cholate was 19.2 mg./100 cc. The average plasma cholesterol and cholate concentration in twenty-five normal subjects was 228 and 5.3 mg./100 cc. respectively. These findings were

of interest because elevation of cholate in animals has been found by us to induce hypercholesteremia.

In order to study this relationship further, nephrosis was induced in rats. Plasma cholate rose in ten rats from 6.2 to 26.2 mg./100 cc., one week after injection of anti-kidney serum. Plasma cholesterol rose from 64.3 to 279.6 mg./100 cc. Since a similar degree of "hypercholatemia" induced in a normal rat by intravenous injection of cholate leads to a cholesterol rise comparable to that found above, it would appear that the "hypercholatemia" observed in nephrotic rats is the cause of their hypercholesteremia.

The oral administration of 100 mg. of cholate/day for 3 days to a series of nephrotic rats also demonstrated this mechanism. The plasma cholate of cholate-fed nephrotic rats was three times higher than that of the control nephrotic rats. The plasma cholesterol concentration also changed in the same direction, being twice as high in the cholate fed series as in the control series.

The discovery of a "hypercholatemia" in both patient and animal having nephrotic hypercholesteremia, together with evidence suggesting that the cholesterol rise in the animal is due to this "hypercholatemia," adds support to the hypothesis that clinical and experimental states of hypercholesteremia are dependent in some manner upon blood cholate content.

Delayed Cutaneous Hypersensitivity in Sarcoidosis. GEORGE J. FRIOU, Newington, Conn. (Introduced by Robert H. Green).

Delayed intracutaneous reactions to oidiomycin, trichophytin, and mumps antigens were studied in twenty-two patients with proven sarcoidosis and sixty-four controls. Fifteen cases of sarcoidosis and twenty controls were tuberculin negative. The antigens used, to which most individuals react, are immunologically distinct from each other and from tuberculin.

Positive tests were less frequent and generally smaller in the sarcoidosis group than among the controls, and this was particularly true of tuberculin negative sarcoidosis. Sarcoidosis cases with negative tuberculin reactions had a significantly smaller mean number of positive tests per case than tuberculin negative controls or tuberculin positive sarcoidosis. There was no appreciable difference between tuberculin positive sarcoidosis and corresponding controls. Five cases of sarcoidosis and no controls failed to react to any antigens.

Considered individually, oidiomycin and mumps antigens gave significantly less frequent positive tests in tuberculin negative sarcoidosis than in corresponding controls. The mean diameters of positive oidiomycin reactions in these groups differed significantly in the same direction. In individuals in these groups with a history of mumps the same difference was observed with mumps antigen. For trichophytin the difference between these groups was just significant when mean diameters for all cases tested were compared.

It was concluded that the decrease in delayed hypersensitive reactions in sarcoidosis is not limited to tuberculin, but is a broad, immunologically non-specific phenomenon probably affecting, when present, all delayed cutaneous hypersensitive reactions. These results remove some support from the theory that sarcoidosis is of tuberculous etiology, since it is usually assumed that the decreased reaction is specific for tuberculin. They indicate that delayed cutaneous reactions are not reliable in specific etiologic studies of sarcoidosis. With this simple technique it is possible to evaluate more completely the significance of a negative tuberculin reaction in this and other conditions.

The Circulatory Consequences of Pneumoperitoneum in Pulmonary Emphysema. ROBERT H. FURMAN, THOMAS M. BLAKE and MILDRED T. STAHLMAN, Nashville, Tennessee. (Introduced by Hugh J. Morgan).

An increasing experience with pneumoperitoneum has suggested that benefit may accrue the patient which is difficult to assess by common pulmonary function tests. Utilizing cardiac catheterization, seven severely emphysematous subjects with low immobile diaphragms were studied at rest and after exercise. Resting output values were low and showed variable but slight change after pneumoperitoneum. Exercise before pneumoperitoneum produced a fall in cardiac output in five, no change in one and a rise in another. No fall was noted after exercise after treatment. Two subjects whose outputs fell after exercise before pneumoperitoneum showed no change after exercise after treatment; output rose in five. Exercise in every instance evoked maximal ventilatory responses. The post-pneumoperitoneum improvement in the cardiodynamic response to exercise was associated with an increased oxygen consumption and extraction and smaller A-V oxygen difference than that noted following exercise before treatment. Increased intrapleural-intraperitoneal pressure differentials and probably improved intrapulmonary gas mixing appear to be the responsible mechanisms. The same mechanisms may obtain in the familiar squatting posture seen after exercise in patients with Tetralogy of Fallot.

Effect of Thyrotropin (TSH) on Adreno-Cortical Function. J. LESTER GABRILOVE, New York, N. Y. (Introduced by Louis J. Soffer).

Thyrotropin (TSH) was administered to three groups: 1) primary hypothyroidism, 2) hypopituitarism (Sheehan's Syndrome), and 3) euthyroidism. Studies included determination of the basal metabolic rate, the serum protein bound iodine level (SPI), the urinary excretion of the neutral 17-ketosteroids and 11 oxygenated corticoids, the circulating eosinophile level, exophthalmometric readings, and serial skin biopsy. Patients with primary myxedema to whom TSH was given failed to demonstrate any clinical improvement nor was there noted any alteration in the BMR, SPI, or urinary steroid excretion. Following the administration of TSH to the patients with Sheehan's syndrome, there was noted clinical and laboratory improvement of the hypothyroidism, concomitant with a decrease in the urinary excretion of the 11

oxygenated compounds and the precipitation of adrenal insufficiency. The urinary excretion of the neutral 17-ketosteroids remained unaltered. In one euthyroid subject, the administration of TSH was followed by a rise in the BMR and SPI and a slight decrease in the urinary excretion of the 11 oxygenated compounds. The urinary excretion of the neutral 17-ketosteroids remained unchanged.

In the skin of patients with Sheehan's syndrome, there was a deposition of metachromatic staining material presumably mucoprotein in nature, similar to that observed in patients with primary hypothyroidism. This substance differed from that encountered in normal skin in its more intense staining characteristics and its localization. The administration of TSH to patients with Sheehan's syndrome or the administration of thyroid extract to subjects with primary hypothyroidism resulted in a decrease in the skin content of the mucoprotein-like material.

Effect of Aureomycin Upon Nitrogen Balance and Urinary Riboflavin Excretion in Undernourished Men. GEORGE J. GABUZDA, GEORGE G. JACKSON and MARGARET E. GRIGSBY, Boston, Massachusetts. (Introduced by Maxwell Finland).

Aureomycin was administered to three undernourished men maintained on constant diets providing 75 gm. of protein and 2800 calories daily. Each patient was studied during three periods: initial control (6 to 9 days), aureomycin administration (2.5 to 3.0 gm. orally daily for 9 to 12 days), final control (9 to 12 days). None of these patients developed diarrhea, stomatitis or glossitis. Anorexia occurred but constant food intakes were maintained.

During the initial control period and the first four days of aureomycin two patients gained and one maintained weight. All patients lost weight during the latter part of the aureomycin period and the first several days of the final study period. Thereafter all patients regained weight.

Average daily urinary nitrogen excretions during the initial control periods were 6.6, 7.1, and 5.5 gm., and nitrogen balances + 4.7, + 3.8, and + 5.5 gm. daily in the three patients, respectively. Aureomycin administration was followed after 1 to 6 days by increases in urinary nitrogen. Average daily nitrogen excretions during aureomycin were 8.5, 10.9, and 7.1 gm., and nitrogen balances + 2.3, − 0.2, and + 3.7 gm. daily, respectively. Urine nitrogen excretions returned to initial levels during the final control period. Stool nitrogen excretions were not significantly altered during these studies.

Urinary riboflavin (microbiologic assay) increased 2.5-fold during aureomycin administration, and preceded the increases in urinary nitrogen by 1 to 5 days. In the final study period riboflavin excretion first decreased to less than the initial control value and then gradually increased again almost to control levels. Stool riboflavin excretion decreased during aureomycin.

In vitro, aureomycin does not invalidate the microbiologic assay for riboflavin.

Serial stool cultures revealed no consistent quantitative or qualitative alterations.

Conclusion: Administration of aureomycin to three patients was associated with loss of weight and an increase in urinary nitrogen and riboflavin excretions.

Changes Suggesting Emphysema in Physiologic Respiratory Measurements in Patients with Neuromuscular Disorders, and in Healthy Individuals. MORTON GALDSTON, New York, N. Y. (Introduced by J. Murray Steele).

Patients suffering from atrophy of thoracic, pectoral and abdominal muscles as a result of neuromuscular disease were found to exhibit some disturbances in physiologic measurements commonly noted in instances of chronic obstructive pulmonary emphysema. These are pulmonary distension (absolute and relative increase in residual air volume), decreased vital capacity and maximal breathing capacity and an abnormally large respiratory dead space volume. Certain seemingly healthy subjects studied over a period of several years have manifested similar physiologic disturbances except for decreased maximal breathing capacity. Some of the apparently healthy subjects also exhibit an increased portion of mixed venous blood which fails to be oxygenated in the lungs and a decreased alveolar diffusing capacity for oxygen. All of these disturbances together are present in patients suffering from chronic emphysema.

The physiologic alterations in emphysema are believed to reflect loss of pulmonary elasticity and destruction of parenchymal tissue due to chronic cough and bronchial obstruction. The fact that none of the patients studied had a history of respiratory complaints or exhibited any of the physical or roentgenographical signs of emphysema makes it doubtful that they have what is generally considered to be emphysema. The evidence suggests that disease of respiratory muscles can bring about pulmonary distension. Even in seemingly healthy individuals unrecognized impairment of action of respiratory muscles or lack of mobility of the thoracic cage can give rise to similar changes. The presence of such unsuspected physiologic derangements in certain individuals may account for instances of seemingly rapid development of pulmonary insufficiency following respiratory infections.

Effect of Combined Cortisone and Penicillin Therapy on Experimental Streptococcal Pneumonia. ROBERT J. GLASER, and LENORE H. LOEB, St. Louis, Missouri. (Introduced by Carl G. Harford).

Recent studies from this laboratory have demonstrated that the course of experimental hemolytic streptococcal pneumonia is more fulminating in rats pretreated with cortisone than in untreated control animals. On the other hand, in a number of human diseases, including several of bacterial origin, cortisone exerts a favorable effect on some signs and symptoms even though the basic pathologic process may be unaffected.

The latter observation raises the question as to whether cortisone might constitute a useful adjunct in the treat-

ment of constitutional manifestations of certain infections for which effective antibacterial agents are available. In order to obtain evidence bearing on this point, the effect of combined penicillin and cortisone therapy in experimental streptococcal pneumonia in rats was studied.

Various experiments were performed in which rats were inoculated by the intrabronchial route with hemolytic streptococci. One group of animals was given cortisone for five days prior to inoculation and subsequently treated with penicillin. Control rats received no cortisone but were given identical courses of penicillin. The cortisone group exhibited less favorable survival rates and increased numbers of purulent complications in comparison with the control group.

When, however, treatment with cortisone and penicillin was begun after inoculation, the infections appeared to be, if anything, less severe than those in control animals receiving penicillin alone. The lesions observed in systematic histologic study of the lungs of rats receiving combined therapy were less intense than those seen in the lungs of the penicillin controls.

Since cortisone modifies the inflammatory response to bacterial infection, in those infections for which effective chemotherapy is available to control growth of organisms *per se*, the use of cortisone to limit inflammation and the concomitant toxic manifestations, which are often severe, may be of value. The results reported herewith suggest that such a possibility merits consideration.

Atrio-Septo-Pexy: A New Surgical Treatment for Atrial Septal Defect. Hemodynamic Studies in a Case Before and After Surgery. HARRY GOLDGERG, CHARLES P. BAILEY, GEORGE D. GECKELER and WILLIAM LIKOFF, Philadelphia, Pennsylvania. (Introduced by Joseph R. DiPalma).

A new surgical treatment for atrial septal defect is presented. The chest is opened by a right 4th interspace incision. Following opening of the pericardial sac, a purse-string suture is placed about the right atrial appendage. Incision is made in the appendage and the left ungloved forefinger introduced into the right atrium. The finger is advanced into the chamber and through the defect. With the finger in-situ the right atrial appendage is sewn to the edge of the defect. By this means the residual cavity of the right atrium is gradually rendered no longer in communication with the left. Following the atrio-septo-pexy, the right atrial appendage is ligated.

Hemodynamic studies were made in a case of interatrial defect before and after the above operation. A 38 year old woman with a murmur since birth presented dypsnea, cardiac enlargement, rough systolic murmur in third left intercostal space, accentuated 2nd pulmonic sound, enlarged pulmonary arteries, hilar dance and increased vascular markings. The ECG showed right heart strain. During cardiac catheterization, the catheter was passed through an interatrial defect into the left atrium. The pulmonic blood flow was 17.3 l/m; systemic blood flow 4.1 l/m indicating a left to right shunt of 13.0 l/m. The pulmonary arterial pressure was 90/40 mm Hg and brachial arterial oxygen saturation 94%. Following surgery, the systolic murmur was inaudible. Though the pulmonary arteries were still enlarged, the hilar dance was less marked.

Recatheterization, 17 days post-operatively, revealed: Pulmonic and systemic blood flows 5.9 l/m; pulmonary arterial pressure 48/33 mm Hg; brachial arterial oxygen saturation 82%. The latter was thought to be due to pulmonary factors subsequent to surgery rather than to a right to left shunt. The surgical procedure eliminated the left to right shunt in the atrium and reduced the pulmonary arterial pressure by 30%.

The Effects of Cortisone on the Reaction to Bacterial Endotoxins and Infections: Imitation by Agents Which "Blockade" the R. E. System. ROBERT A. GOOD, FLOYD W. DENNY, JR. and LEWIS THOMAS, Minneapolis, Minnesota.

Previous studies showed that a single injection of gram-negative bacterial toxin causes bilateral renal cortical necrosis in cortisone-treated rabbits. This lesion can only be produced in normal rabbits by giving *two* intravenous injections of toxin spaced 24 hours apart (the generalized Shwartzman reaction). The observation suggested that a defense mechanism which protects normal rabbits against toxin is impaired by cortisone treatment. In view of the known capacity of cortisone to cause dissolution of lymphoid tissues, the possibility that defense might involve cells of the reticuloendothelial system was considered.

Beeson showed that R. E. "blockade" with thorotrast or trypan blue enhances susceptibility to the local Shwartzman reaction. Experiments were undertaken to determine whether these substances have an effect comparable to that of cortisone on the response to one injection of toxin.

A single injection of meningococcal or *S. marcescens* toxin, 6 hours after an injection of thorotrast, regularly produced the following results: 1) The lethal effect of toxin was enhanced 500-fold. 2) Bilateral cortical necrosis of the kidneys occurred in a high percentage. 3) An intradermal injection of toxin caused local skin hemorrhage as well as renal cortical necrosis.

Identical results were obtained when trypan blue was used for "blockade." Neither thorotrast nor trypan blue caused illness or tissue lesions when given alone, or when given a few hours after, instead of before, toxin.

The previously demonstrated capacity of cortisone to enhance streptococcal infection was similarly imitated by thorotrast. When streptococci were injected a few hours after thorotrast, extensive and uniformly fatal septicemia occurred.

The results suggest that interference with a protective function of the R. E. system may be the basis for the effects of cortisone on bacterial infection and intoxication.

The Effect of Glucose Diuresis on the Renal Excretion of Sodium Bicarbonate. A. V. N. GOODYER, L. G. WELT, J. H. DARRAGH, W. A. ABELE and W. H. MERONEY, New Haven, Conn. (Introduced by Arthur J. Geiger).

The urinary excretion of bicarbonate is thought to be the result of partial rejection, in the distal tubules, of that

small (about 15%), constant fraction of filtered bicarbonate which escapes reabsorption in the proximal tubules. Any increase in this fraction would augment the urinary excretion of bicarbonate, provided distal tubular activity remained constant.

It was therefore of interest to determine whether glucose diuresis (probably mediated in the proximal tubules) would alter the rate of excretion of bicarbonate when distal tubular reabsorption of bicarbonate was minimal.

A high rate of bicarbonate excretion was induced in three hydropenic normal subjects by the steady infusion of a 1.4% solution of sodium bicarbonate. When urine flow and the excretion of bicarbonate and chloride were constant, 750–1000 cc. of a 25% solution of glucose was administered.

Urine flow and the excretion of sodium chloride increased greatly as glycosuria supervened. Nevertheless, the excretion of bicarbonate *decreased* as much as 50%. The excretion of potassium increased from low values in one experiment, and decreased from high values in two. Glomerular filtration was unchanged or increased slightly.

The decrease in bicarbonate excretion was correlated with the dilution of serum bicarbonate, chloride and sodium by water withdrawn from cells as a result of the hyperglycemia. Serum pH also decreased slightly.

The data are interpreted to indicate that a decrease in serum bicarbonate (or pH) may be the stimulus that increases the tubular reabsorption of bicarbonate in spite of a large proximal tubular diuresis.

The data emphasize the priority of influences originating in the internal environment (concentration of bicarbonate or pH of serum) over local renal factors (proximal tubular diuresis) in the regulation of the rate of excretion of bicarbonate.

The Importance of a 'Sodium Retaining Factor' in the Urine in the Mechanism of Edema Formation. EDGAR S. GORDON,* JEROME J. CHART and ELVA S. MEYER, Madison, Wisconsin.

Presence in the urine of an antidiuretic factor, possibly posterior lobe pituitary hormone, has been demonstrated in various types of clinical conditions involving fluid accumulation. The cause for and the mechanism of this phenomenon is unexplained. Using a new, sensitive biological assay method for a sodium retaining factor in urine, high titers of this material have been found consistently in clinical patients exhibiting edema and fluid retention. There is striking correlation between these titers and fluid and sodium balance. Toxemia of late pregnancy, cardiac decompensation, renal disease with edema and hepatic cirrhosis have been studied. The factor under study is believed to be steroidal in nature and probably of adrenal origin. A working hypothesis has been developed for the mechanism of abnormal fluid accumulation from various causes in which altered adrenal function with sodium retention is believed to be primary, followed by increased antidiuretic hormone formation.

Studies of Pulmonary Distention. FRANK D. GRAY, JR.* and FRIEDA G. GRAY, New Haven, Connecticut.

The functional residual capacity of the lung (FRC), a measure of the total lung volume at the end of a normal expiration, reflects the degree of expansion or distention of the resting lung. In general, factors tending to impair passive expiration increase the distention of the resting lung and diminish alveolar ventilation.

In an effort to determine some of the factors influencing pulmonary distention studies were undertaken of the FRC as affected by various therapeutic measures including vaponefrin inhalation, digitalization of patients with chronic pulmonary disease or mitral stenosis, and administration of cortisone or ACTH to patients with rheumatoid arthritis. The last group had, in some cases, evidence of spondylitis. Finally, vaponefrin and cortisone therapy were studied in a patient with Boeck's sarcoid.

As was expected vaponefrin reduced the FRC in proportion to the degree of bronchospasm present. In patients with chronic pulmonary disease, digitalization produced an increase in FRC in some and a decrease in others with no discernible pattern. In the mitral stenosis group digitalis regularly increased the FRC. The increase was roughly related to the degree of pulmonary artery hypertension developing after digitalization. Cortisone or ACTH in rheumatoid arthritis patients resulted in a dramatic and consistent decrease in FRC paralleling the increase in skeletal mobility. A patient with severe Boeck's sarcoid treated with cortisone showed a marked increase in FRC followed by a decrease toward normal. An initial increase was also seen following vaponefrin. In this case the drugs may have initially opened collateral respiratory channels thereby overcoming atelectasis, then relieved bronchial obstruction thus finally decreasing distention.

It was concluded that bronchial obstruction, pulmonary vascular engorgement, and immobility of the chest cage may produce reversible pulmonary distention.

The Stomach as an Endocrine-like Organ. Factors Influencing Urinary Uropepsin Excretion. SEYMOUR J. GRAY, JOHN A. BENSON, JR., ROBERT W. REIFENSTEIN and COLIN RAMSEY, Boston, Mass. (Introduced by Clifford L. Derick).

Uropepsin is a proteolytic enzyme present in the urine of healthy persons in constant amounts. Its origin in the stomach has been established by the disappearance of the enzyme following total gastrectomy. The urinary level of uropepsin is considered a measure of the endocrine-like activity of the stomach resulting from the secretion of pepsinogen into the blood stream by the peptic gland.

Studies were undertaken to investigate the role of the stomach as part of a hypothalamic-pituitary-adrenal-gastric pathway. ACTH and cortisone administered over a period of 3 to 4 weeks produced a marked increase approximating 200% in the basal and nocturnal gastric secretion of acid and pepsin and a similar increase in urinary unropepsin excretion. In every instance adrenocorticotropic hormone increased the acid secretion and uropepsin excretion to the active duodenal ulcer level.

The adrenal steroids including Compound F produced a uniform and standardized increase in uropepsin excretion approximating 150–200% in normal subjects within 24–48 hours. This hormonal response appears to be independent of vagal control and parallels the circulating eosinophiles in the blood. Similar results have been obtained in patients after vagus section.

The urinary uropepsin excretion in patients with Cushing's disease (with adrenal hyper-function) and in patients with brain tumors involving the hypothalamus was elevated significantly. Diminished levels were observed in patients with Addison's disease and hypopituitarism. Exceedingly low levels were found in 80 patients with pernicious anemia.

The stress of surgical procedures and burns appears to produce identical increases in the uropepsin excretion parelleling the fall in circulating eosinophiles. Other stress factors such as physical exertion, anoxia, shock and x-ray irradiation elicit similar responses.

The relationship of hormonal influences to the gastric responses to stress as part of a hypothalamic-pituitary-adrenal mechanism will be discussed.

Permeability Studies on the Blood Cerebrospinal Fluid Barrier in Polyneuritis. MARK A. GRIFFIN, Philadelphia, Pennsylvania. (Introduced by George D. Gammon).

The penetration of the dye, fluorescein, into the spinal fluid after intravenous injection has been studied in cases of polyneuritis in which the cerebrospinal fluid protein is high. Normally this dye cannot be detected in spinal fluid after intravenous injection but in the neuritis cases showing a protein of 100 mg. per ml. the dye was readily detectable in amounts roughly proportional to the protein level. This demonstrates an increased penetration of this substance and suggests that some at least of the protein may be derived from this serum through increased permeability of meningeal and vascular surfaces. This concept, if correct, might explain certain of the clinical features of the disease.

Hexamethonium Salts Alone or with 1-Hydrozaline or Regitine for Patients with Hypertension. K. S. GRIMSON,* Durham, N. C.

Tests in animals and man reveal that hexamethonium bromide, chloride, bitartrate, and citrate administered parenterally produce hypotension with minimum tachycardia, interrupt vaso-motor reflexes and delay transit time of barium; actions resembling those of the ganglionic blocking agent, Etamon. Single oral doses of one gram of the bromide, chloride, or bitartrate produce minimal effects. However, during the first several days evidence of effectiveness developed in each of 60 patients now continuing treatment, two to six grams daily, as long as 17 months. Most patients have relief of symptoms, some have moderate and a few marked reduction of blood pressure.

1-Hydrozaline, Apresoline, initially tested and given to patients in 1948 occasionally reduced blood pressure when 100 mg. were given intravenously or as much as 250 mg. orally. Continuing oral treatment was discontinued because of little reduction of pressure and occurrence of side effects. Regitine, an imidazoline derivitive, was tested in 1949 using 60 or 120 mg. doses orally. It produced more reduction of pressure but continuing treatment was discontinued because of tachycardia. Apresoline or Regitine has now been given to those of the 60 patients taking hexamethonium daily, whose blood pressure has not been satisfactorily reduced. Some additional reduction of blood pressure occurred. Tachycardia has been less frequently troublesome. Of 7 of these patients now tested by intravenous injections of 50 mg. of Regitine slight increase of pulse rate occurred in 5 and typical marked increase in only two.

The Renal Excretion and Extrarenal Uptake of Histidine and of Total Amino Acids in Man, and the Effect of ACTH and Cortisone Administration. DAVID GROB, Baltimore, Md. (Introduced by F. B. Bang).

The factors responsible for the increased histidinuria induced by ACTH or cortisone administration were investigated by study of the mechanism of renal excretion of endogenous and injected histidine, the renal and extrarenal components in the disappearance of injected histidine, and the influence of ACTH and cortisone on these processes. Histidine was found to be normally excreted by a mechanism involving tubular reabsorption of approximately 93 per cent of filtered histidine, with no limitation of transfer capacity in the range of tubular loads studied. The rate at which the injected compound disappeared from the extracellular fluid by means other than renal excretion was 10 to 30 times the rate of disappearance through the kidneys. The administration of ACTH or cortisone produced a three-fold increase in the rate of urinary excretion of endogenous or injected histidine, which was attributable primarily to a decrease in tubular reabsorption to approximately 80 per cent of filtered compound. Increased glomerular filtration played a lesser role. The rate of extrarenal uptake of injected histidine was reduced by 30 to 50 per cent during hormone administration, but this could account for only part of the increased excretion. It is suggested that the diminished extrarenal and tubular transport of histidine induced by ACTH or cortisone may reflect a widespread decrease in the cellular transport of this compound.

The increased excretion of total amino-acid nitrogen induced by ACTH or cortisone was also attributable in large part to decreased tubular reabsorption. The data indicate that part of the increased total nitrogen excretion and negative nitrogen balance induced by these hormones is renal in origin.

Protein Synthesis by Human Colon Mucosa. CHARLES M. GROSSMAN, JAMES D. HAUSCHILDT and JAMES D. CASE, Portland, Oregon. (Introduced by Arthur W. Frisch).

There is abundant evidence that in-vitro incorporation of radioactive amino acids into protein reflects protein

synthesis. We have found sigmoid colon mucosa to incorporate significant amounts of carboxyl-labeled glycine into its protein. Colon mucosa strips from surgical resections were washed and incubated with 0.33 microcuries of labeled glycine. Proteins were precipitated, lipid extracted, and radioactivity measured. Controls were obtained by precipitating at zero time. Results are expressed as specific activities (S. A.—counts per minute per milligram dry protein).

S. A. of colon protein ranged from 65 to 1175. Zero time mean ± S. E. was 6.3 ± 1.3. Incorporation was regularly depressed by penicillin but not by aureomycin. Inhibition by nitrogen or cyanide was marked. Omitting calcium or magnesium from the buffer gave inconsistent results in contrast to consistent depression with liver slices. Homogenized tissue incorporated insignificant amounts of labeled glycine, yet good bacterial growth was demonstrable. Slices of colon smooth muscle yielded protein with S. A. 45, compared to 350 with mucosa from the same patient. Mucoprotein was isolated and found to have significant S. A., although invariably lower than total protein from the same tissue. S^{35} inorganic sulfate was found to be incorporated into both total protein and mucoprotein. The S^{35} was easily removed from total protein by solution in alkaline urea and reprecipitation, whereas the C^{14} label was not removed by this procedure. This may be explained by coprecipitation of mucoprotein with total protein.

These findings indicate that the distal colon mucosa is an active membrane participating in metabolic functions other than absorption and storage. The data suggest that in addition to synthesizing mucoprotein the cells are capable of incorporating labeled glycine into other protein moieties.

The Effects of Subtotal Adrenalectomy Combined with Sympathectomy Upon the Cerebral Vascular Resistance of Patients with Severe Hypertension. JOSEPH H. HAFKENSCHIEL and CARL K. FRIEDLAND, Philadelphia, Penna. (Introduction by William A. Jeffers).

Blood flow to the brain was measured by the nitrous oxide method in six patients before, and at intervals ranging from three to fifteen months after operation. Mean changes from the preoperative to the initial postoperative study were: Mean arterial pressure 174 to 114 mm. Hg, cerebral vascular resistance 3.7 to 2.1 mm. Hg per cc. blood per 100 grams brain per min., cerebral blood flow 50 to 63 cc. per 100 grams per min., cerebral oxygen uptake 3.8 to 4.1 cc. per 100 grams per min., arterial carbon dioxide tension 42 to 39 mm. Hg, jugular venous oxygen tension 32 to 37 mm. Hg. The last is assumed to represent minimal cerebral oxygen tension. The only significant changes were the reductions in blood pressure and cerebral vascular resistance. These were highly significant ($p < 0.01$). Five of six patients showed cerebral vascular resistances above the mean (2.8 ± 0.7) for a large series of hypertensive patients. After adrenalectomy, four of the six had resistance values below that of Kety's normals (1.6 ± 0.4), the other two having decreased from 4.6 to 3.9 and from 3.7 to 2.7 respectively.

Three patients were tested again three to thirteen months after the first post adrenalectomy study. The time studied after adrenalectomy did not appear to influence the degree of resistance change. These observations suggest that the increased cerebral vascular tone of these patients has been reduced by sympatho-adrenal surgery without alterations in cerebral blood flow, oxygen uptake or tension.

Observations on Body Composition and Body Water Metabolism in Normal Pregnancy. HAROLD B. HALEY and JOHN W. WOODBURY, Boston, Massachusetts. (Introduced by C. Sidney Burwell).

Deuterium was used to measure total body water in pregnant women.

Equilibrium time after injection of D_2O was the same in three patients in midpregnancy as in non-pregnant women. Cord blood, placental tissue, colostrum and milk had the same deuterium concentration as blood serum.

Total body water was determined in three women at four to six-week intervals during the last two-thirds of pregnancy. Each patient gained weight, water, and solids. In the three weeks before delivery, the average gain of water was 1.5 liters (range 0.9–2.1); of weight, 0.9 kilograms (range 0–1.5). This difference indicates differential accumulation of water over solids.

Body water determinations were made in five patients during the first twelve hours post-delivery and one week later. All lost body solids. (Average loss 1.3 kilograms; range 0.9–2.0). Four lost both weight and water; one gained weight by accumulating water. The loss of solids is interpreted as due to (1) lochia, (2) involution of hypertrophied organs, (3) postpartum nitrogen loss possibly analogous to the metabolic response to trauma.

The disappearance rate of D_2O from the blood, and therefore from the body water, measures the water turnover rate or the balance between total intake and total output. The three patients studied before delivery had a normal turnover rate during the prenatal period. Of six patients studied in the first week postpartum, five showed a high water turnover rate with an abrupt change to lower levels at about the fourth postpartum day.

Thus at the end of normal pregnancy, these women selectively retained water. In the immediate postpartum period, there was an increase in water turnover rate and these rates fell to normal about the fourth day. In the first postpartum week there was a loss of body solids and in most patients a net loss of water.

Studies in the Mode of Action of Chemotherapeutic Drugs in Bacterial Endocarditis. MORTON HAMBURGER* and LEON STEIN, Cincinnati, Ohio.

The success of penicillin in the treatment of subacute bacterial endocarditis, in contrast with the ineffectiveness of sulfonamides has often been attributed to ability of the former and inability of the latter to penetrate fibrin vegetations. The indifferent success of the "broad spectrum" antibiotics has been ascribed to their bacteriostatic rather than bactericidal action.

In an attempt to throw light upon these hypotheses, experiments of Liebold and Nathanson were modified and extended, using a strain of streptococcus viridans from a case of endocarditis, and employing penicillin G, sodium sulfathiazole, aureomycin, terramycin, and chloramphenicol as test drugs. The streptococcus was seeded in fibrin clots prepared from human Fraction I, in petri dishes, and in brain heart infusion agar. Filter paper discs saturated with different concentrations of the test drugs were placed upon the surface, following which they were incubated at 37 C for 24 hours.

The results were as follows:

(1) Zones produced by all test drugs, over a wide range of concentrations, were usually wider on fibrin plates than on agar plates.

(2) Colonies were seen under low power magnification in the microscopic clear zone produced by sulfathiazole and broad spectrum antibiotics, but not in penicillin zones.

(3) Cultures from the *surface* of penicillin zones were sterile in all concentrations tested. Cultures from the *depths* of high concentration penicillin zones were negative after 1 week's incubation; from moderate concentration zones negative at 24 hours but positive in 48; from low concentration zones positive after over-night incubation. In contrast, culture from the depths of *all* broad spectrum antibiotic and sulfathiazole zones were positive, and even from the surface were negative in only the highest concentrations employed.

The experiments suggest that the bactericidal action of penicillin in the depths of a vegetation is probably its most important characteristic in determining its effectiveness in bacterial endocarditis.

A Hemolytic Mechanism in Pernicious Anemia Influenced by B_{12}. HENRY E. HAMILTON, RAYMOND F. SHEETS and CLINTON D. JANNEY, Iowa City, Iowa. (Introduced by Elmer L. DeGowin).

Eight patients with pernicious anemia were transfused with fresh blood from normal donors of heterologous, compatible A-B-O group and Rh type. The concentrations of donors' erythrocytes during periods up to 120 days were measured by serial counts of inagglutinable cells by a modification of Ashby's technique yielding accurate data for mathematical analysis.

Analysis of the disappearance curves of normal transfused cells showed normal survival with B_{12} started 9 and 12 days before transfusion. In patients beginning treatment 9 days before and 1, 4, and 5 days after transfusion, random destruction of the normal erythrocytes, irrespective of age, was demonstrated. In general, the rate of loss increased the later B_{12} was given, with the fastest rate in the untreated patient.

In six studies the rate of random cell destruction was constant throughout the four-month period, despite coincidentally adequate reticulocyte responses and satisfactory rises in total erythrocyte counts. In two studies exhibiting the fastest rate of destruction the rate accelerated about 70 days after transfusion.

Conclusions: (1) Fresh normal erythrocytes are so damaged in a few days in the milieu of the human body deficient in B_{12} that they are destroyed in random fashion, presumably by the trauma of circulation. (2) The cell damage incurred in the first few days is not reversible by the subsequent administration of B_{12}. (3) When severe grades of erythrocyte damage have occurred, the chemical changes of aging or the trauma of 70 days of circulation further hasten destruction. (4) These studies indicate that 45 micrograms of B_{12} weekly must be present in the body for at least 10 days before a safe environment is provided for normal erythocytes. Until further evidence is available, it is assumed that the hemolytic mechanism exerts similar effects on the circulating erythrocytes of the patient.

Biosynthesis of Nucleic Acids Studied in Unicellular Systems. LEONARD HAMILTON, GEORGE BOSWORTH BROWN and C. CHESTER STOCK, New York, New York. (Introduced by David Karnofsky).

As a biological model for studying polynucleotide synthesis in a single species of mammalian cells (such as human leukocytes) as distinct from overall synthesis in intact mammals, the incorporation of C^{14}-labelled purines into the nucleic acids of *Paramecium aurelia* was studied. Both "killer" (51–7K) and "sensitive" (51–75B) varieties of *P. aurelia* utilize adenine-8-C^{14} for the synthesis of nucleic acid adenine and guanine; the nucleic acid adenine and guanine show the same proportionate uptake of C^{14}; 2,6-diaminopurine-2-C^{14} is converted into nucleic acid guanine and adenine but preferentially to the former. The purine utilization patterns differ from those of *Aerobacter aerogenes* on which paramecium feeds. *A. aerogenes* utilizes adenine for the synthesis of polynucleotide adenine and guanine with preferential transformation to the former, 2,6-diaminopurine and to a greater extent guanine-8-C^{14} are used preferentially for the formation of polynucleotide guanine and comparatively little for transformation into polynucleotide adenine. Another protozoan, the chrysomonad flagellate *Ochromonas*, incorporates adenine twice as readily into polynucleotide adenine as into guanine; it utilizes guanine and 2,6-diaminopurine for both polynucleotide adenine and guanine with preferential incorporation into the guanine. These patterns of purine incorporation can be compared with these published for other species: *L. casei* in which adenine and guanine serve equally well as precursors of polynucleotide adenine and guanine; the rat in which adenine is utilized for polynucleotide adenine and transformed into guanine but in which guanine is only slightly utilized; *Tetrahymena geleii* in which adenine is utilized for polynucleotide adenine but not transformed into guanine whereas guanine is utilized for both polynucleotide guanine and adenine. Similar studies are now in progress on the biosynthesis of nucleic acids in normal and leukemic human leukocytes.

Effects of Restriction of Dietary Fat and Cholesterol Upon Serum Lipid Patterns in Patients with Hypertension. FREDERICK T. HATCH and FORREST E. KENDALL, New York City, New York. (Introduced by David Seegal).

Serum lipid patterns, including free and total cholesterol, lipid phosphorus and neutral fat have been determined in hospitalized patients with severe hypertension on four levels of dietary fat and cholesterol intake. Forty patients were studied on a normal fat (85 gm. per day) and cholesterol (1 gm. per day) intake and then on a cholesterol-free diet containing 3 gm. of fat per day (Kempner rice diet). Groups of these patients were also studied on fat intake levels of 50 to 60 gm. per day (12 patients) and 20 to 40 gm. per day (10 patients). Protein and salt intakes constituted uncontrolled variables in these studies, but other experiments indicated that their influence on serum lipids was not important.

During the period of normal fat and cholesterol intake the serum lipid levels of the patients did not differ significantly from those of healthy subjects of the same age. On a fat intake between 50 and 60 gm. per day the levels were not significantly altered. With restriction of dietary fat to below 40 gm. per day, changes in the serum lipids were observed which varied greatly among individuals. Although total cholesterol level was decreased significantly in 68% of the patients, there were significant increases in 12% of the series. Eighteen per cent of the patients developed a lipemia characterized by turbid serum, slight elevation of free cholesterol and substantial elevations of lipid phosphorus and neutral fat levels.

Ultracentrifugal serum lipoprotein studies performed in twelve of these patients by Dr. Gofman revealed that the rice diet reduced the S_f 12–20 complex level in only one patient; substantial *rises* occurred in five patients.

It is concluded that drastic dietary fat restriction in patients with hypertension evokes changes in the serum lipids which are highly variable among individuals. Whereas two-thirds of patients exhibit decreases in total serum cholesterol, nearly one-fifth of patients develop lipemia and nearly one-half show increases in S_f 12–20 lipoproteins.

Vitamin B_{12} Levels in Pernicious Anemia. JOHN W. HARRIS, Boston, Mass. (Introduced by William B. Castle).

Employing Euglena gracilis *var.* bacillaris for Vitamin B_{12} assay, it was consistently possible to detect concentrations of 6 $\mu\mu$g/ml and to recover 70–90% of crystalline material added to normal or pernicious anemia sera. "Free-B_{12}" was assayed directly on sterile samples; "Total-B_{12}" was assayed on samples previously heated at 100° C for one hour—this procedure having been found to make available to the organism the maximum B_{12} activity.

Ten normal sera averaged 23 $\mu\mu$g free-B_{12}/ml and 55 $\mu\mu$g total-B_{12}/ml; normal erythrocytes, 36 $\mu\mu$g total-B_{12}/gm; and normal peripheral blood or bone marrow buffy coats, 831 $\mu\mu$g total-B_{12}/gm.

Sixteen sera from pernicious anemia patients in relapse contained no detectable free-B_{12} and averaged 1 $\mu\mu$g total-B_{12}/ml; erythrocytes showed no detectable B_{12} activity; but peripheral blood or bone marrow buffy coats averaged 121 $\mu\mu$g total-B_{12}/gm.

Sera from three patients with megaloblastic anemia but with free HCl in gastric juice contained 82, 60, and 32 $\mu\mu$g total-B_{12}/ml.

Sera from five groups of pernicious anemia patients maintained on different amounts of crystalline B_{12} or liver extract given intramuscularly every 4 weeks were studied. The sera were obtained 4 weeks after the last injection of a series lasting eighteen months. The average total-B_{12}/ml of individual patients from each group was as follows: Group I, followed as relapse rate controls on saline injections alone, 6 $\mu\mu$g B_{12}/ml; Group II on 5 μg B_{12}, 18 $\mu\mu$g/ml; Groug III on liver extract assayed to contain 5 μg B_{12}, 25 $\mu\mu$g/ml; Group IV on 15 μg B_{12}, 31 $\mu\mu$g/ml; and Group V on liver extract assayed to contain 15 μg B_{12}, 32 $\mu\mu$g/ml.

The Effect of Hemorrhage and the Pyrogenic Reaction on Hepatic Circulation in Dogs. HENRY HEINEMAN, CHEVES MCC. SMYTHE and PAUL A. MARKS, New York, N. Y. (Introduction by Stanley E. Bradley).

The effect of rapid hemorrhage on hepatic circulation and hepatic cellular function was studied in 12 dogs anesthetized with nembutal. The hepatic blood flow (EHBF) was estimated as the bronsulfalein (BSP) clearance and BSP extraction was determined directly using the technique of hepatic venous catheterization. The arterial pressure was measured directly with a strain gauge and recording galvanometer.

The average control EHBF was 31 ml/kg body weight/min. Immediately following rapid phlebotomy of 1.3 to 3.9% of body weight, EHBF fell to 28 to 80% of control levels in 10 of 12 dogs. There was a concomitant drop in mean arterial pressure of 20–59 mm Hg. During this period of decrease in EHBF there was an increase in hepatic vascular resistance. Associated with these changes there was an increase in arterial-hepatic vein oxygen difference and an increase in BSP extraction by the liver. These data suggest that hepatic vasoconstriction occurred immediately following hemorrhage.

During spontaneous recovery, EHBF rose to 71 to 99% of control values within 20 to 50 minutes following blood loss. This "recovery" of EHBF occurred although the arterial blood pressure consistently remained lower than control levels and did not tend to rise.

In two animals there was no decrease in EHBF following hemorrhage. Both had pyrogenic reactions prior to phlebotomy and EHBF increased significantly during this reaction and persisted elevated through the post-hemorrhagic period.

The effect of pyrogen (typhoid vaccine) on EHBF was studied further in four dogs. Within 10 to 20 minutes after pyrogen was administered EHBF increased 32 to 119% above control values, with a decrease in BSP extraction by the liver.

Dynamic Aspects of Carbon Dioxide and Urea Metabolism in the Human Studied with Radioactive Carbon. LEON HELLMAN and MAXWELL L. EIDINOFF, New York, N. Y. (Introduced by Konrad Dobriner).

Radioactive carbon (C^{14}) labelled precursors have been used to study the quantitative aspects of the formation of urea from carbon dioxide in the human. The rates of synthesis and the total amounts (pools) of carbon dioxide and urea present in the body have also been determined.

Bicarbonate labelled with C^{14} was administered intravenously and the expired labelled CO_2 was collected at short time intervals. Simultaneous urine collections were made and the specific activity of the C^{14} labelled urea, which could only have been derived from the administered C^{14} bicarbonate, was determined. Over 80% of the administered bicarbonate was eliminated as CO_2 within 8 hours. The CO_2 specific activity curve reveals an initial rapid mixing of the labelled bicarbonate with the body fluids, followed by a decrease in activity which reflects the rate of production and the size of the carbon dioxide pool of the body. In a 70 kg man, the CO_2 pool renews itself or turns over approximately once each hour and consists of 30 Grams of carbon dioxide, chiefly in the form of bicarbonate.

The mathematical analysis of the curves for urea and carbon dioxide is in accord with the concept that the carbon of urea is directly derived from the carbon dioxide pool. The urea pool consists of approximately 12 Grams of urea distributed in a volume equal to the total body water and turns over once every 12 hours. The size and turnover rate of the urea pool calculated above has been confirmed in an independent manner by administering labelled urea and measuring the extent to which it is diluted by the urea present in the body.

This relationship between CO_2 and urea was shown to be a general one by comparing excreted CO_2 and urea activities after the administration of C^{14} acetate and methionine which form carbon dioxide in the course of their metabolism.

Rapid Changes in Plasma P.B.I. Levels in Euthyroid and Hyperthyroid Subjects. BASIL S. HETZEL, DOROTHY S. DE LA HABA and LAWRENCE E. HINKLE, JR., New York, N. Y. (Introduced by Harold G. Wolff).

The plasma protein bound iodine (P.B.I.) has been shown to be a measure of the blood thyroid hormone and is being used for the diagnosis of thyroid disease. In this investigation hitherto unsuspected fluctuations have been found in association with exposure to stressful life experiences.

The alkaline ash method of Barker has been used and found to give satisfactory results so that deviations of greater than $\pm 12\%$ have been significant. Serial observations at hourly intervals have been made over 3 to 7 hour periods in euthyroid and hyperthyroid patients who had not previously ingested iodine in any significant amounts. Under neutral conditions the P.B.I. has been observed to be remarkably constant during the period of observation in both euthyroid and hyperthyroid subjects. However, during experiences of stressful significance to the individual marked fluctuations have been found—rises of as much as 100% one to three hours afterwards in some subjects, or falls of up to 60% in others.

The pertinence of these findings to Graves' disease is currently under study. However, it is concluded that the possibility of these changes should be taken into account in using the P.B.I. as an aid in clinical diagnosis.

Normal and Impaired Retinal Vascular Reactivity to Oxygen and its Correlation with Cerebral Vascular Reactivity to Carbon Dioxide. JOHN B. HICKAM,* JAMES F. SCHIEVE and H. O. SIEKER, Durham, North Carolina.

It is known that normal retinal arteries and veins will constrict when the arterial blood oxygen tension is increased and dilate when it is lowered. The present study was undertaken to determine whether this reaction is altered in persons with known vascular disease, and whether retinal vascular reactivity (RVR) to this stimulus can be correlated with some measurement of cerebral vascular reactivity (CVR).

Eyeground photographs were made while the subjects breathed first air and then tank oxygen. The diameter of the larger retinal vessels was measured from the photographs, using a low-power microscope with a scale. RVR was expressed as per cent shrinkage in vessel diameter while breathing oxygen. CVR was measured as increase in cerebral blood flow (cc/100 grams brain/min.) resulting from inhalation of 5% carbon dioxide.

Studies of RVR were made on 24 normal persons, 21 hypertensives, and 14 diabetics. In addition, RVR and CVR were measured together in 17 persons. Normal persons (ages 16 to 83) showed a slight decline in RVR with advancing age. The arteries of the hypertensive and diabetic patients showed significantly less reactivity ($P < .01$) than normal. The veins of the diabetics also showed less reactivity ($P < .05$) than normal. There was a significant correlation ($r = .793$) between reactivity of the retinal arteries to oxygen and increase in cerebral blood flow on 5% carbon dioxide. There was no correlation between reactivity of the retinal veins and CVR.

It is concluded that the retinal arteries usually undergo significant loss of their reactivity to oxygen in patients with hypertension and diabetes, and that this loss is well correlated with loss of the ability to increase cerebral blood flow in response to carbon dioxide. The loss of RVR in patients with disorders affecting the vessels, and the simplicity of the technique, suggest that the subject deserves further study.

Isolation and Concentration of Human Blood Platelets: Their Properties in vitro and in vivo. ERWIN O. HIRSCH, FRANK H. GARDNER and E. DONNALL THOMAS, Boston, Massachusetts. (Introduced by Samuel L. Gargill).

Whole blood was centrifuged at 4° C in plastic containers at $400 \times G$ for 15 minutes. Supernatant plasma

containing 50 to 90% of the platelets and as little as 2% of the white cells was siphoned into plastic containers, spun at 1,000 × G for 10 minutes, and the platelets then washed twice with resuspension between washings. Preparations containing 1,000,000 platelets per cu. mm., 600 white cells and practically no red cells can be routinely obtained in this fashion.

When .066 M sodium citrate (one part to nine parts of blood) was used as anticoagulant and one-hour old platelet-rich plasma was infused into a patient with aplastic anemia, platelets survived normally. After being washed twice with .066 M sodium citrate platelets did not appear to be viable *in vivo*. *In vitro* they produce clot retraction and normal clotting time in thrombocytopenic plasma. Serum prothrombin time, however, is lengthened far beyond that of fresh plasma containing the same number of platelets.

When 1% disodium ethylene-diamine-tetraacetate was used as an anticoagulant and platelets were washed twice with saline, serum prothrombin time was the same as that of fresh plasma containing the same number of platelets.

Washed platelets exhibit oxygen consumption and utilize glucose. They produce CO_2 and lactic acid. Platelets from a patient with scurvy exhibited normal oxygen consumption. On storage, platelets lose their ability to cause clot retraction within 24 hours. Their effect on clotting time and serum prothrombin time persists for weeks. Oxygen consumption decreased rapidly and was always zero after three days' storage.

Spermine and Tubercle Bacilli. JAMES G. HIRSCH, New York, New York. (Introduced by Gardner Middlebrook).

In previous studies a substance inhibiting the growth of mammalian tubercle bacilli *in vitro* was isolated from tissues in crystalline form. When added to a modified Dubos medium, a final concentration of 0.001 per cent (0.01 mg. per cc.) of this crystalline material completely inhibited the growth of several strains of human and bovine mycobacteria, regardless of the size of the bacillary inoculum. Other microorganisms, including avian tubercle bacilli, saprophytic mycobacteria and various non-acid-fast bacteria, were much less susceptible or completely resistant to the inhibitory action of this substance.

After a variety of chemical studies, the crystalline material was identified as the phosphate salt of an organic base, spermine. This polyamine (NH_2—$(CH_2)_3$—NH—$(CH_2)_4$—NH—$(CH_2)_3$—NH_2) has been demonstrated to occur widely in animal tissues, including those of man. Little is known concerning the origin, function and fate of the spermine present in the body.

Recent investigations inquiring into the mechanism of action of spermine on tubercle bacilli reveal that suppression of growth occurs only if an unidentified component of certain sera, serum fractions and aqueous extracts of some organs is present in the system. This activating substance is a protein with the characteristics on an α globulin. Very small quantities of this protein, much less than 1 microgram per cc., bring about the full inhibitory action. Further studies of the nature of this activator and of the mechanism by which it produces, in conjunction with spermine, a toxic effect on tubercle bacilli are in progress.

Effects of Head-up Tilting, Pitressin, ACTH, Cortisone and Desoxycorticosterone Glucoside on Excretion of Sodium and Chloride. BERNARD C. HOLLAND, Durham, North Carolina. (Introduced by Eugene A. Stead, Jr.).

Motionless standing causes retention of water, sodium and chloride. Previous studies have shown that the retention of water is related to activity of pituitary ADH mechanism, but the factors responsible for the salt retention remain unknown. The effect of head-up tilting at angle of 45° was compared with the effect of administration of pitressin, ACTH, cortisone and desoxycorticosterone glucoside.

Normal subjects were placed on a diet containing 24 gm. of sodium chloride per day. After a 12-hour fast, they were given 1 gm. of NaCl in 200 cc. of water each hour for six hours. Subjects either remained horizontal without medication or at the end of the second hour were tilted or given medication.

Tilting uniformly caused a marked decrease in excretion of sodium, chloride and water without a significant fall in eosinophiles.

Pitressin in doses of 3 milli-units per kilogram caused no change in excretion of sodium and chloride, and no fall in eosinophiles.

Cortisone (200 mgm. orally) or ACTH (20 mgm. i.v.) had no consistent effect on excretion of sodium. Chloride excretion tended to rise. There was marked eosinopenia.

Desoxycorticosterone glucoside (50 mgm. i.v.) caused an increase in chloride excretion without a consistent effect on sodium excretion. The eosinophiles did not fall.

Neither pitressin, cortisone, ACTH nor desoxycorticosterone glucoside causes in the first four hours the pattern of sodium and chloride retention that is produced by head-up tilting. Tilting causes marked salt retention without a marked fall in eosinophiles.

Role of Tissue Binding in Local Erythroid Maturation Following Instillation of Vitamin B_{12} into Marrow Cavity. DANIEL L. HORRIGAN and ROBERT W. HEINLE,* Cleveland, Ohio.

A local erythroid maturation effect following instillation of vitamin B_{12} directly into marrow cavity of persons with pernicious anemia was described previously. The interpretation of this observation was that the vitamin was capable of inducing maturation without first being altered.

Other investigators have shown that vitamin B_{12} exists in the blood in bound form, that many substances are capable of binding, and that the unbound vitamin is ineffective in causing erythroid maturation *in vitro*.

In this study, the observations indicate that the vitamin is bound at the site of instillation but that binding capacity

is limited. After instillation of 1 mcg. into the marrow cavity of three patients, previously reported, and of 0.25 mcg. in another, maturation occurred only at the site of injection without other hematologic response. In two patients, however, instillation of 1 mcg. was followed by a systemic response, and generalized marrow maturation, although the latter was more advanced at the site of instillation.

These findings suggest that in the patients who showed local maturation only, the vitamin was completely bound at the injection site, whereas in those having a systemic response, only part was bound locally, the remainder being available for systemic utilization. Local binding capacity is thought to be related to the degree of tissue vitamin deficiency and varies in different patients. That the effective binding substance and gastric intrinsic factor are the same has been suggested but not proven. If combination with a binding substance is necessary before vitamin B_{12} becomes active, a deficiency of tissue binding substance might be the basic defect in certain refractory macrocytic anemias associated with megaloblastic or abnormal normoblastic bone marrow states as have been described in cirrhosis and certain other conditions.

Viremia in Poliomyelitis Following Infection by a Natural Route. DOROTHY M. HORSTMANN,* New Haven, Conn.

For the past 40 years viremia in human poliomyelitis has been considered to be of rare occurrence, and unimportant in the pathogenesis of the disease. These conclusions have been based on the largely negative results of attempts to demonstrate the virus in the blood of patients. In fact, only one positive result has been reported, and this with blood drawn from an abortive case within several hours of onset. However, the negative results may have been due to the search for virus having been made too late in the course of the human disease, and for this reason the problem was attacked experimentally in cynomolgus monkeys (*M. Cynomolgus*) and chimpanzees (*Pan satyrus*) following infection by a natural route, i.e. orally. In 4 separate experiments, animals which had been fed Y-SK or Egypt strains of poliomyelitis virus (Lansing and Brunhilde types, respectively) were bled from the femoral vessels daily or every other day for 5-7 days following virus feeding. Blood specimens from the chimpanzees and from the monkeys which subsequently became paralysed, were then tested for virus by intracerebral inoculation into rhesus monkeys. Three of 4 chimpanzees and 9 of 12 monkeys to whom virus had been fed in this manner were thus demonstrated to have poliomyelitis virus in the blood between the 4th and 6th days after virus ingestion; in some animals on all 3 consecutive days. The chimpanzees remained asymptomatic, but in the case of the monkeys, viremia *preceded* the onset of paralysis by 3 to 7 days.

These results in experimental animals reopen the possibility that viremia may occur in human poliomyelitis. It seems likely that it may occur during the incubation period of the human infection, or in association with the minor illness or "first phase" of the disease.

A Model of Plasma Iron Kinetics in Human Subjects. REX L. HUFF,* PAUL ELMLINGER, ROBERT MORTIMER, HAL ANGER and C. A. TOBIAS, Berkeley, California.

On the basis of total plasma iron, body surface counting of iron 59, and plasma iron 59 data, a kinetic model has been hypothesized. The data are from forty patients with hematopoietic disease and from seven normal subjects. They were given single intravenous injections of iron 59 attached to globulin-IV-7 (Cohn). The model provides the basis for rate computation for the movement of plasma iron. The plasma data of the normal subjects were analyzed into a polynomial of three decaying exponentials having half times of approximately 0.1, 0.5, and 3 days. The initial component accounted for dilution in a quantity of iron approximately forty times that of the plasma iron. This represents iron in equilibrium with that of the plasma and possibly is some fraction of storage iron as well as that in pigment forming tissues—chiefly those tissues forming hemoglobin. The subsequent dilution of tracer in this equilibrium pool is thought to be related to the incorporation of the iron into pigment. The appearance of a third slow component may represent the turnover of storage iron.

The data of the patients differed in several ways, some of which required a more complicated model than that of the normal subjects. The graphic analysis of some patients' data resulted in polynomials of more than three exponentials. Half times of the first component of a magnitude compatible with complete portal clearance of the tracer correlated with *in vivo* data showing primarily hepatosplenic turnover, similarly half times of the first component compatible with complete bone marrow clearance of tracer correlated with *in vivo* data indicating marrow accumulation. Liver storage of iron 59 and depression of erythropoiesis was uniformly associated with a long half-time of the first component as well as a decreased amplitude.

Homologous Transplantation of Human Kidneys. DAVID M. HUME, JOHN P. MERRILL and BENJAMIN F. MILLER,* Boston, Massachusetts.

Homologous transplantations of human kidneys have been studied in six patients. The recipients were patients in far-advanced, irreversible, chronic uremia. Donor kidneys were obtained during surgical procedures in three instances, and from cadavers in the remainder.

The transplanted kidneys were placed in the recipient's thigh, with anastomosis of renal artery to the profunda branch of the femoral artery and renal vein to the common femoral vein. The ureter was brought out through the skin, permitting collection of urine directly from the transplanted kidney.

The periods of ischemia of the donor kidneys lasted from 14 minutes to 205 minutes. The transplanted kidney which had been ischemic for the longest period functioned better than the others, but no conclusion can be drawn from such a small series of observations. The phenolsulphonthalein test on this kidney indicated 22% excretion in 2 hours as against 0% in the bladder urine. On

the 21st post-operative day, clearances on the transplant were obtained: *inulin*, 14 cc/min/1.73 M^2; *urea*, 6.4 cc/min.; *creatinine*, 19 cc/min.; *renal plasma flow*, 112 cc/min. During the several weeks that this transplant functioned, the blood urea nitrogen was reduced from 130 to 40 mgms%.

Three transplanted kidneys never developed measurable function because of technical difficulties. The other three kidneys went through a period resembling acute tubular necrosis, with anuria for 8 to 14 days, following which they produced urine, attaining in one instance a volume of 2800 ml daily. Two transplants functioned considerably longer than any homotransplants reported in animals.

Infection was the chief difficulty encountered. It eventually destroyed all the transplants. It is possible that concomitant administration of cortisone and ACTH may have intensified these infections.

A Dextrorotatory Urobilin from Feces Following Antibiotic Therapy. G. WATSON JAMES, III, ANNE ANDERSON and VIVIAN WILKERSON, Richmond, Virginia. (Introduced by Bernard M. Jacobson).

Stercobilin, strongly levorotatory, has been isolated from feces by Watson, and urobilin IX, *a*, optically inactive, has been prepared synthetically from mesobilirubinogen, but is also present in small quantities in some pathological urines. Both of these compounds have been thoroughly described. A dextrorotatory urobilin in infected bile, and in feces of a patient following aureomycin has been reported, but not isolated in quantities for adequate study.

From the feces of patients with various hemolytic anemias, stercobilin was isolated. Then aureomycin or terramycin was given for several days and urobilinogen formation depressed. When the Ehrlich reaction had returned the feces were subjected to the same isolation procedure, and now a crystalline dextrorotatory urobilin, different from stercobilin was regularly obtained in large quantities. It was still present in the feces for as long as sixty days after the antibiotics were discontinued. In one patient with N^{15} labelled red cells the heavy isotope content of the stercobilin and *d*-urobilin were the same suggesting a common source. During chloromycetin administration only stercobilin was obtained.

d-Urobilin possesses some of the chemical and physical properties of both urobilin IX, *a*, and stercobilin. Two crystal forms have been observed. The chemical reactions are more similar to those of urobilin IX, *a*, and oxidation to ferrobilin and glaucobilin is easily performed. The Ehrlich reaction of *d*-urobilinogen is both quantitatively and qualitatively the same as stercobilinogen. Other properties, a possible structural formula, and its significance will be discussed.

The Effect of a Single Injection of ACTH upon Urinary Excretion of Sodium, Potassium and 17-Ketosteroids. WILLIAM MCK. JEFFERIES, ALFRED K. BOCHNER and RALPH I. DORFMAN, Cleveland, Ohio. (Introduced by Joseph M. Hayman).

Adrenal cortical activity is manifested by effects which can be divided into three main categories: (1) regulation of electrolyte metabolism, characterized by a retention of sodium and a loss of potassium (DOCA effect), (2) regulation of carbohydrate metabolism (S hormone effect), and (3) an androgenic or protein anabolic effect (N hormone effect). The current test of adrenal cortical response to 25 mgm. ACTH i.m. involves changes in circulating eosinophiles and urinary excretion of uric acid, both reflections of changes in S hormone effect, only one of the 3 phases of action.

The effect of administration of 25 mgm. ACTH i.m. upon urinary excretion of sodium, potassium and 17-ketosteroids in specimens collected according to the same schedule as the Thorn test was studied in 10 normal young men. In every instance an increase in excretion of K and 17-ketosteroids occurred, whereas a decline in excretion of these substances was seen in three patients with Addison's Disease tested in the same fashion. In 8 tests urinary Na excretion decreased, but decreases were also seen in the Addisonians. Control studies indicated that these effects could not be attributed to changes in urine volume or to diurnal variation. Following a single alarming stimulus, the change in excretion of these substances was found to correspond well to that following a single injection of ACTH. A case of adrenogenital syndrome showed a lack of increase in excretion of K with a good increase in excretion of 17-ketosteroids following a single injection of ACTH.

Hence, by modifying the Thorn test to include determinations of changes in urinary excretion of Na, K and 17-ketosteroids, a more complete reflection of the pattern of response of the adrenal cortex to a single stimulus may be determined.

Hepatitis in Mice of Presumed Viral Origin. JANET JORDAN, CHARLES I. LEFTWICH and GEORGE S. MIRICK,* Baltimore, Maryland.

The saline extract of the liver of a patient (CB) who died of hepatitis twenty-seven days after exposure, was injected intraperitoneally into mice given 0.1% urethane to drink. Monthly serial intraperitoneal transfers of organ extracts were made in three week old urethanized mice. In the fourth passage 2 mice developed a disease characterized by ascites, hepatomegaly and splenomegaly. Microscopic sections of the liver showed central and periportal mononuclear cell infiltration and occasionally central or diffuse necrosis. Eleven subsequent positive monthly serial transfers have been completed. Organ extracts and ascitic fluid diluted to 1/1000 and blood were infectious when inoculated parenterally but experimental, oral and intranasal inoculations were negative. The incubation period is 3–4 weeks. Most mice survive with anatomical restoration of the liver and the appearance of some immunity. Many control series of mice were negative prior to the isolation of the agent in the laboratory. Since that time the disease has occasionally been seen in uninoculated control mice as well as control passage series inoculated with human material thought to

be normal. Rabbits, guinea pigs, hamsters, monkeys and embryonated eggs inoculated with material infectious for mice remained well. Bacteriological cultures have been negative and the available evidence indicates that the disease is not due to a virus of the psittacosis group or to LCM, PVM, the mouse hepatitis virus of Gledhill and Andrewes, the agent of Ehrlich's ascitic tumor, leptospira, or to ectromelia. Streptomycin, terramycin, chloramphenical and sulfadiazine do not inhibit the disease. All attempts to neutralize the agent with convalescent serum from patients with hepatitis, γ globulin and convalescent mouse serum have failed. The agent has resulted in gastro-intestinal symptoms and abnormal liver function tests when inoculated into volunteers.

Transmission of Acute Non-Bacterial Gastroenteritis to Volunteers: Evidence for Two Different Agents. WILLIAM S. JORDAN, JR.,* IRVING GORDON, RICHARD G. HODGES and WILLIAM R. DORRANCE, Cleveland, Ohio.

Clinical and epidemiological data suggested that at least two types of infectious gastroenteritis, afebrile and febrile, occur in families. Studies comparing the febrile type with a known strain (Marcy) are reported here. Bacteria-free supernate of stool from a febrile case (FS-I-7549) produced anorexia, abdominal cramps and loose stools in four of eight volunteers after 26 to 30 hours. Three of the four experienced vomiting and fever.

The illnesses induced by the febrile inoculum differed from those induced by the Marcy strain, a filterable agent obtained from an institutional outbreak. When fed as stool supernates, this agent has produced afebrile illnesses characterized chiefly by watery diarrhea.

Supernates from sixth human passage Marcy and first passage FS stools were fed to volunteers in individual isolation. Six of seven men given 7 ml Marcy became ill within 24 to 120 hours. One of eight given 2 ml FS developed a temperature of 101.8, nausea, anorexia and headache in 20 hours; five noted mild abdominal symptoms after 20 to 30 hours. Sixteen days later, the men ingested the same inocula, but the amount of FS was increased to 10 ml. No illnesses occurred in the Marcy group. In the FS group, one definite illness occurred at 30 hours; two other men had mild symptoms.

Thirteen days later, cross immunity was tested. Marcy inoculum induced diarrheal disease in seven of eight men, including two previously sick with FS. FS inoculum induced febrile disease in three of seven men, all previously sick with Marcy, within 21 to 30 hours.

Lack of heterologous immunity and the different clinical pictures produced suggest that one of the types of gastroenteritis observed in families, the febrile type (FS), differs from the Marcy type and presumably represents a new entity.

Further Studies on the Antidiuresis and Decrease in Sodium Excretion During Venous Congestion of the Limbs: Its Prevention in Normal Subjects by a Large Transfusion; Its Absence or Presence in Cardiac Patients with or without Congestive Failure. WALTER E. JUDSON, J. DONALD HATCHER, MEYER H. HALPERIN, and ROBERT W. WILKINS,* Boston, Massachusetts.

In previous reports to the Society it was shown that:

1. Antidiuresis and decreased sodium excretion, and usually decreased renal blood flow, occur during venous congestion of the limbs in normal subjects and in hypertensive patients before and after splanchnicectomy.

2. Decreased sodium excretion *without* antidiuresis may be produced by venous congestion of the limbs in normal subjects under osmotic (mannitol) diuresis, or in patients with diabetes insipidus.

In the present work it was found that:

1. Antidiuresis and decreased sodium excretion do *not* occur during venous congestion of the limbs of normal subjects who concomitantly are given a large (1 to 1.5 L.) blood transfusion, but may occur if a smaller volume of blood is given. This suggests that a decrease in the effective circulating blood volume, and not peripheral factors such as venous distention, edema formation or discomfort in the limbs, is the important stimulus to the antidiuretic response.

2. Antidiuresis and decreased sodium excretion may occur during venous congestion of the limbs in certain patients with severe heart disease.

3. Antidiuresis and decreased sodium excretion do not occur during venous congestion of the limbs in certain other patients with severe heart disease and congestive failure.

4. Measurements of cardiac output (Fick) and of pulmonary arterial, right ventricular, and peripheral arterial pressures during venous congestion of the limbs in selected normal and cardiac subjects suggest that antidiuresis occurs when there is a decrease in cardiac output or peripheral arterial pressure, but does not occur when there is no decrease in cardiac output or peripheral arterial pressure, even though there may be decreases in pulmonary arterial and right ventricular pressures.

Ascorbic Acid Requirements in "Damage" and Its Relationship to Adrenocortical Activity. ROBERT M. KARK,* ROBERT E. CHAPMAN and C. FRANK CONSOLAZIO, Chicago, Illinois.

Levenson et al. (Ann. Surg. *124:* 840, 1946) have shown precipitous falls in urinary ascorbic acid in "damaged" surgical patients during the "catabolic phase" of stress, despite parenteral therapy with large (normally saturating) amounts of ascorbic acid. These data are accepted widely as evidence that requirements for ascorbic acid are increased after surgery and other insults. The demonstration, in rats, that adrenocortical activity consumed adrenal ascorbic acid during and after "stress" offered a seemingly rational explanation for the so-called increased utilization of ascorbic acid.

This study reports effects of therapy with adrenocorticotropin or cortisone on: the plasma, whole blood, fecal and urinary ascorbic acid; eosinophils; and urinary 17-ketosteroid excretion, in patients and healthy individuals, who lived on fixed dietary regimens and fixed (analyzed) ascorbic acid intakes, in a metabolic unit. Available data

showed that: (a) In classical scurvy, good adrenocortical response to A.C.T.H. was indicated by drop in eosinophils and rise in 17-ketosteroid excretion. Ascorbic levels in blood, urine and feces did not change. (b) In individuals "saturated" with ascorbic acid, urinary ascorbic acid increased considerably during A.C.T.H. or cortisone therapy and during adrenal rebound. Blood, urine and fecal ascorbic acid levels did not change. (c) In "unsaturated" individuals change in urinary ascorbic acid was variable but retention of urinary ascorbic acid did not occur.

The data indicate that in man: consumption of ascorbic acid by the adrenal for synthesis of cortical hormone cannot be considerable; adrenocortical activity cannot account for urinary retention of ascorbic acid seen after surgery; diuresis of ascorbic acid occurs with adrenocortical activity when large amounts of ascorbic acid are ingested; perhaps comparable with adrenocortical glycosuria.

On the basis of the above and studies in patients given A.C.T.H. after surgery, speculations will be made on ascorbic acid metabolism in the catabolic phase of "damage."

The Effect of Various Bacterial Derivatives and of Physical Injury on the Lysozyme-like Activity of Human Leukocytes. GRACE P. KERBY, Durham, North Carolina. (Introduced by D. T. Smith).

Using an in vitro method of assay of a lysozyme-like enzyme present in human leukocytes, an increased enzymatic activity has been demonstrated on exposure of the leukocytes to various bacterial derivatives (Proteus vulgaris, Ps. aeruginosa, E. coli, B. subtilis and Friedlander B carbohydrates; meningococcal filtrate; pneumococcal type III SSS). In some instances (Proteus vulgaris, Ps. aeruginosa, E. coli and B. subtilis carbohydrates) the per cent rise in activity over the control has tended to be measurably proportional to the amount of substance added. In other instances, the method has not differentiated amounts of the substance added, within the range tested. The enzymatic activity of leukocytes as measured varies from day to day in the same individual and from individual to individual, but increased activity in the presence of the test bacterial derivative as compared to the control has been consistent.

The enzymatic activity is increased more markedly by lysis of the leukocytes by saponin or distilled water than by exposure to the bacterial derivatives. Also, progressive decrease in the concentration of a suspending saline solution below isotonicity produces progressive increase in enzymatic activity of the suspended leukocytes. The possibility is considered, therefore, that the increased activity noted in the presence of the bacterial derivatives reflects damage to the leukocyte wall, with release of enzymes into the suspending medium.

The findings to date suggest that the method may be adaptable to a gross, in vitro titration of various substances such as the bacterial pyrogens which are damaging to leukocytes. Studies are in progress also to determine the effect of the bacterial derivatives on the leukocytes of individuals with bacterial hypersensitivities and with induced pyrogen tolerance. The usefulness of the method as a means of determining the metabolic intactness of stored leukocytes remains to be clarified; preliminary studies suggest this possibility.

Paradoxical Effects of Cortisone on Influenza B Virus Infection of the Chick Embryo. EDWIN D. KILBOURNE, New Orleans, Louisiana. (Introduced by Thomas M. Rivers).

Investigations pursuant to previously described observations of increased virus concentrations in fertile hen's eggs injected with cortisone have revealed in eggs so injected a diminution or delay in the toxic effects of Influenza B virus multiplication concomitant with the attainment of allantoic fluid virus concentrations equal to or far exceeding those of controls. Decreased toxicity was manifested by increased survival time and lower allantoic fluid protein concentration of eggs administered cortisone.

The paradoxical effects of increased survival time and increased virus concentration observed with the administration of the same dose of cortisone have been partially clarified by a study of time-dosage relationships. The intra-allantoic injection of chick embryos with 1 mg. of cortisone 1–20 hours after their inoculation with Influenza B virus (Lee strain) results in no significant modification of survival time and in greatly increased virus concentrations. Injection of the same amount of cortisone 24–42 hours subsequent to the initiation of infection induces a significant increase in survival time in the presence of concentrations of virus equal to or slightly exceeding those of controls. In contrast, injection of cortisone 18–24 hours prior to viral inoculation has resulted in the earlier death of cortisone-treated embryos.

Studies of Antibiotic Antagonism. WILLIAM M. M. KIRBY* and JAMES J. AHERN, Seattle, Washington.

Jawetz has shown that chloramphenicol interferes with penicillin both *in vitro* and *in vivo*. In the animal experiments, effective antibiotic blood levels were present for only a few hours, and the results reflected the interference of chloramphenicol with the early bactericidal action of penicillin. The slower, but progressive, killing action of the combination, which often leads to sterilization of cultures *in vitro*, played little if any role. It seemed desirable, therefore, to repeat the experiments under conditions resembling those used in clinical therapy.

Mice were infected with group A streptococci, and chloramphenicol and a long-acting form of penicillin were injected repeatedly over a 3 day period. Under these circumstances, interference was not demonstrable. These results were interpreted as being due to the continued bactericidal action of the combination and, in addition, to the fact that time was allowed for effective phagocytosis to occur.

A 31 year old man with subacute bacterial endocarditis was treated with antibiotics for 3 years, relapsing when-

ever therapy was stopped. The infecting organism, a viridans streptococcus, was killed *in vitro* by a combination of penicillin and chloramphenicol, and the patient was cured when these two antibiotics were administered for 25 days.

Twenty-five patients with pneumococcal pneumonia were treated with penicillin and 25 with penicillin and aureomycin, and the clinical course of the 2 groups was compared. No evidence of interference was observed.

Interference would appear to be of clinical importance only when recovery depends upon the early killing action of penicillin. Lepper and Dowling observed interference of aureomycin with penicillin in pneumococcal meningitis. Rapid killing is probably important in this instance because surface phagocytosis is relatively inefficient in the subarachnoid space.

It is concluded that interference of broad spectrum antibiotics with penicillin is seldom of clinical importance, and that under certain circumstances these combinations are desirable.

Some Effects of Hexamethonium on Renal Circulation. WALTER M. KIRKENDALL and JAMES W. CULBERTSON, Iowa City, Iowa. (Introduced by Willis M. Fowler).

Eight patients had differential renal function studies before and after intravenous administration of hexamethonium chloride (C-6) in doses of 20 to 160 mgm. Two patients were feebleminded but otherwise normal, one had malignant hypertensive cardiovascular disease, two had coarctation of the aorta, and three had moderate essential arterial hypertension. Glomerular filtration rate (GFR) was measured by inulin and renal plasma flow (RPF) by p-aminohippuric acid plasma clearance. Usually three clearance periods were determined before and after administration of hexamethonium. All patients developed upright postural hypotension following injection, demonstrating effective autonomic ganglionic blockade.

In one normotensive person, the two patients with aortic coarctation, and the one with malignant hypertension renal plasma flow rates fell from 11 to 24 (av. 19)% and glomerular filtration rates fell from 14 to 25 (av. 18)% below control values. The filtration fraction (FF) was not significantly altered in any subject.

In the second normotensive subject and all three patients with essential hypertension RPF increased from 13 to 75 (av. 45)%. There was little change in GFR, but FF decreased from 24 to 47 (av. 32)% below control values.

Apparently the effect of hexamethonium on renal circulation differs in health and certain diseases. In one normotensive person without arteriolar spasm, in coarctation of the aorta (where the renal circulatory pattern may not be maintained by increased neurogenic tone), and in malignant hypertension with irreversible structural damage in the kidney, hexamethonium depressed RPF and GFR without changing FF significantly. In the other normotensive person, who had a "hypertensive" renal pattern before administration of hexamethonium, possibly from apprehension, and in three essential hypertensives changes in renal hemodynamics may have followed relaxation of spastic efferent arterioles indirectly from lowering of systemic arterial pressure or directly from release of neurogenic vascular tone in the kidney.

The Mechanism of Diuresis in Diabetic Acidosis. HARVEY C. KNOWLES, JR., Cincinnati, Ohio. (Introduced by Eugene B. Ferris).

The major cause of dehydration in diabetic acidosis appears to be an osmotic polyuria which is accompanied by losses of electrolyte. The present study is concerned with verifying the flow-load relationship in this type of polyuria, and with the composition of the urinary solute load.

The studies concern 22 subjects of ages 20 to 50 years, in ketoacidosis, stuporous, with serum CO_2 contents less than 13 meq/l and plasma urea nitrogen less than 30 mg.%, free from shock and anuria. Urine specimens were collected before and just after the start of treatment. Blood specimens were collected in the pre-treatment period.

The urine flow could not be correlated with the plasma glucose level. There was some correlation between urine flow and glucose excretion. However, the relation between flow and total excreted solute load was found to be essentially linear and highly significant, similar to that observed in normal hydropenic subjects during osmotic diuresis. The mean total urinary osmolarity was found to be 543 M Osmols/L and the mean urinary flow 3.54 cc/min. Analysis of the mean osmolar composition of the urinary loads revealed glucose to be the largest constituent, amounting to 42%. Other constituents were ketones 7%, sodium 6%, potassium 6%, phosphorus 4%, and chloride 2%. Of total nitrogen, urea nitrogen contributed 10%, and the remaining nitrogen, if expressed entirely as ammonia, 19%, of the osmolar composition.

Increased urinary flow was accompanied by increased sodium excretion but little change in potassium or phosphorus excretion.

Conclusions: (1) The diuresis in diabetic acidosis is osmotic in nature, with glucose the main urinary solute. (2) Sodium constitutes only a small part of the solute load; its excretion increases at higher rates of urinary flow.

Reduction of Tetrazolium by Growing Tubercle Bacilli. D. KOCH-WESER, W. R. BARCLAY and R. H. EBERT,* Chicago, Illinois.

The reduction of colorless triphenyl tetrazolium to red formazan has been used as an indicator of the metabolic activity of tissues and bacteria. Previous investigations have been for the most part qualitative. This study reports quantitative metabolic studies of tubercle bacilli in Dubos' liquid medium. 2 mgm. of triphenyl tetrazolium were added to growing cultures of H37RV and after 24 hours the amount of formazan present was estimated colorimetrically after extraction with ethyl acetate. The amount of tetrazolium reduced was directly proportional to the number of bacilli in the culture as determined

turbidimetrically. An excess of asparagine added to the medium markedly increased tetrazolium reduction.

Streptomycin added to the cultures, in a concentration of 50 g. per ml., 6 days after inoculation and at a time when the bacilli were in the logarithmic phase of their growth, immediately decreased the rate of growth and the amount of tetrazolium reduced. Complete inhibition occurred only 3 to 4 days after the antibiotic was added. Once complete inhibition of growth occurred the bacilli did not reduce tetrazolium as long as streptomycin was present in the medium.

Old cultures which had almost ceased growing reduced the same amount of tetrazolium per mgm. of bacilli as did young actively growing cultures.

The reduction of tetrazolium appears to be a useful reaction for studying the metabolism of tubercle bacilli.

The Role of Desoxyribonuclease and a Nuclease Inhibitor from Leucocytes in the Lupus Erythematosus Cell Phenomenon. N. B. KURNICK, L. SCHWARTZ, S. PARISER, S. LEE and W. IRVINE, New Orleans, Louisiana. (Introduced by Albert Segaloff).

The observation of Klemperer et al, that depolymerization of the desoxyribonucleic acid (DNA) of mesenchymal cells is a constant feature in lupus erythematosus, suggests the action of desoxyribonuclease (DNase) in this phenomenon and Hargraves' "L.E. Cell Phenomenon."

The serum DNase activities of a number of normal individuals, patients with acute disseminated lupus erythematosus (L.E.) and patients with other diseases, indicated a wide range of activities for each group. L.E. sera tended to display elevated DNase activities, but there was no correlation between the level of DNase activity, the ability to elicit the L.E. phenomenon, and the severity of the disease. Furthermore, DNase activity, but not L.E. factor activity, is destroyed at 56°.

However, since cell nuclei contain DNase, its relation to the serum L.E. factor was investigated. We found that extracts of normal human WBC contained an inhibitor for serum DNase. The most potent of such inhibitors strongly inhibited the L.E. cell phenomenon, but this effect was not observed with less active inhibitor preparations. Since simple saline extraction removed some inhibitor from the WBC, washed WBC were tested as subtrates for the L.E. cell phenomenon, and found to be considerably more sensitive. Addition of an active inhibitor again suppressed the phenomenon.

Studies aimed at establishing the relationship of the inhibitors of DNase and of the L.E. phenomenon will be reported. The effect of enzymes, dialysis, incubation with normal and pathological sera, and with Fraction II, and salt fractionation will be presented. Attempts at localization of the inhibitors in cytoplasmic and chromosomal fractions of WBC and their species interactions, will be reported.

A hypothesis of the pathogenesis of the L.E. phenomenon and the general significance of the leucocyte DNase inhibitor will be discussed.

The Velocity of Wave Propagation in Muscular Arteries of Normotensive and Hypertensive Subjects. MILTON LANDOWNE,* Baltimore, Maryland.

A new method for the study of pulse wave velocity has provided information relating to the pressure-volume characteristics and elastic properties of the brachioradial artery in living subjects.

Repetitive pressure disturbances are introduced by small impacts delivered at one site along the vessel, being superimposed upon the ambient arterial pressure. These are detected and timed on arrival at another location by a suitable pressure transducer and indwelling needle. Several hundred waves may be recorded in a minute, permitting determination of wave velocity as a function of blood pressure.

A consistent relationship has been obtained in almost all subjects studied. Velocity (v) increases with pressure (p), and this variation usually is larger than the differences between subjects at any single pressure. With rare exceptions dv/dp decreases as pressure increases. In many, but not all individuals, and for grouped information, the experimental data may be closely approximated (a.d $< 2\%$) for convenient representation as v (meters/sec.) $= a\, p^b$ (mm. Hg). Averaged data for 81 normotensive males aged 20–94 yield $a = 2.4$; $b = 0.45$, and for 14 hypertensive patients of various grades of severity $a = 2.0$; $b = 0.50$. The differences are not assigned significance. Marked variation in the degree of estimated medial sclerosis is also without striking effect.

The observations indicate that physiological distensibility of muscular arteries is relatively slight. With a change in arterial pressure from 50 to 150 mm. Hg, the computed maximal increase in vessel diameter is 2.5%. Other evidence supports this value. The estimated elastic modulus of extension of the artery increases with pressure, consonant with the rubber-like properties of fibrillar proteins. Assuming vessel dimensions, a mean absolute value is of the order of 10^6 dynes/cm^2, slightly lower than moduli reported for isolated muscle or elastic fibres.

Quantitative Antibody Studies in Man. Antibody Response in Leukemia and Allied Disorders. DANIEL L. LARSON, New York City, N. Y. (Introduced by Joseph C. Turner).

Antibody responses following the administration of pneumococcus capsular polysaccharides I and II have been studied in patients with leukemia and allied disorders. Antibody determinations were carried out using the quantitative precipitin technique developed by Heidelberger. Results are expressed as micrograms of antibody nitrogen per 4 milliliters of serum.

Patients with chronic lymphatic leukemia and with multiple myeloma exhibited little or no antibody formation. Subjects with chronic myeloid leukemia showed antibody responses somewhat higher than the normal level. Individuals with acute leukemia demonstrated the production of exceedingly large amounts of antibody, and this response was impaired in patients treated with cortisone.

The ability to form antibody to pneumococcus capsular polysaccharides is influenced by the type of leukemia present. Individuals with the highest percentage of immature white blood cells in their peripheral blood and bone marrow had the largest antibody responses. There was no apparent relation between the total white blood cell count in the peripheral blood and the amount of antibody produced. Patients with acute leukemia were noted to have a distinct impairment of antibody response when receiving cortisone.

Some Hormonal Aspects of Water Excretion in Man. ALEXANDER LEAF, H. RASMUSSEN, A. R. MAMBY and M. S. RABEN, Boston, Mass. (Introduced by Walter Bauer).

It has been claimed that anterior pituitary destruction will ameliorate or abolish experimental diabetes insipidus. A patient with diabetes insipidus was observed to lose polyuria and polydipsia spontaneously. Studies revealed insufficiency of anterior and posterior pituitary function. However, the physiologic defect of diabetes insipidus, inability to produce a hypertonic urine during severe dehydration, persisted. The decrease in polyuria reflected simply a decreased total solute load requiring excretion.

Metabolic studies were conducted while the subject was kept on a constant diet. Salt and urea administration caused return of polyuria by increasing the excretory solute load. Thyroxine, desoxycorticosterone, cortisone, corticotrophin (Astwood), and growth hormone (Raben) had no effect upon the renal concentrating ability or water excretion. Hence their alleged diuretic actions are not direct effects but are secondary to their action of increasing metabolism, appetite and the urinary solute load.

The inulin and PAH clearances of this subject were 50% of normal, consistent with panhypopituitarism. With corticotrophin these clearances rose to 75% of normal. Growth hormone, which has been considered to be the renotropic hormone, failed to elevate the renal clearances above the control level when given for twelve days in dosages up to 240 mgs. per day. Balance studies revealed that this absence of effect of growth hormone on renal clearances is to be contrasted with the phosphorous and nitrogen retention that occurred during its administration. These observations with an anabolically active preparation of growth hormone suggests that the renotropic effects of the anterior pituitary are not caused by growth hormone.

Protection of Tuberculin Hypersensitive Cells from Toxic Action of PPD by Cortisone in Tissue Culture. ROBERT H. LEAHY and HERBERT R. MORGAN,* Rochester, N. Y.

Previous investigators have demonstrated that ACTH or cortisone will inhibit the development of the lesions in experimental allergic diseases in animals; this might be due to suppression of: a) humoral antibody formation, b) tissue response to injury, or c) damage to capillaries which accompanies such reactions. It was decided to investigate the role of the response of isolated tissues in culture where the factors of humoral antibody and capillary damage are excluded. Splenic tissue from tuberculin hypersensitive guinea pigs was selected for these experiments, since Rich had demonstrated the cytotoxic action of tuberculin upon macrophages from such tissue.

The roller tube method in tissue culture was used with a fluid medium to which was added: 1) cortisone acetate suspension, or 2) PPD, or 3) both cortisone acetate and PPD. For the first 24 hours culture tubes received plain nutrient medium or the cortisone-containing medium, depending on their subsequent experimental conditions. These media were replaced by nutrient containing PPD or nutrient containing both PPD and cortisone. Daily measurements of migration of macrophages from the splenic fragments were made. Tuberculous tissue grown in nutrient containing PPD alone produced few macrophages, which failed to migrate after the first day and appeared to undergo cytolysis. Tuberculous tissue grown in nutrient containing both cortisone and PPD showed migration and numbers of macrophages equal to that seen with tissue grown in nutrient alone and gave no evidence of cytolysis.

These findings indicate that cortisone acts directly on tuberculin hypersensitive cells *in vitro* to protect them from the cytotoxic action of PPD and therefore that the suppression of humoral antibody formation or protective action against damage to capillaries is not essential for the influence of cortisone on certain aspects of cellular injury in hypersensitivity.

The Relation of Tissue to Serum Acid Phosphatase Concentration in Diagnosis and Treatment of Metastatic Adenocarcinoma. H. M. LEMON, I. ASIMOV, M M. DAVISON, M. K. SCHWARTZ and M. A. DEROW, Boston, Mass. (Introduced by C. P. Emerson, Jr.).

Acid phosphatase is a universal constituent of cell nuclei, but varies widely in concentration in cytoplasm, where large amounts are present only in adult prostatic glands, neurones, certain endocrine glands, and keratinizing epidermis. Quantitative histochemical estimations of acid phosphatase activity of 54 human carcinomas and sarcomas and their normal homologous tissues of origin indicate increased concentrations of enzyme in adenocarcinomas of breast and colon, but not in epidermoid carcinomas. Forty-four observations of 22 colon carcinomas using a previously described technic (Science *109:* 233–235, 1949) showed a mean of 0.145 ± 0.014 (S.E.) mg. P hydrolyzed at pH 5.0 from glycerophosphate per mg. cellular nitrogen per hour, compared with 0.092 ± 0.007 (S.E.) mg. for homologous colon mucosa. Benign uterine tumors and chronic inflammatory lesions did not show any deviation of phosphatase activity from normal. Partial purification of acid phosphatase, yielding a preparation with 3 major electrophoretic components, has been accomplished using prostatic tissue, and an extensive comparison has been made of the enzymatic function of

acid phosphatase from benign hyperplastic and malignant sources.

These tissue observations supplement those made previously on the prostate by Huggins and others, and have an important bearing upon the use of serum acid phosphatase determination in the differential diagnosis of osteolytic bone disease and in evaluation of effects of hormone therapy of neoplasia. This will be illustrated by examples taken from a series of over 30 patients with advanced metastatic adenocarcinoma observed over a period of several years. Since acid phosphatase remains juxta-chromosomal within the nucleus during carcinogenesis, frequently appears to fluctuate in concentration within cells and body fluids according to tumor growth rate and nature of endocrine therapy and has been shown in this work to hydrolyze phosphorylated amino acids, further investigation appears indicated of its relation to malignant cellular degeneration.

Metabolic Fate of Infused Erythrocytes. STANLEY M. LEVENSON,* MARY A. MALONEY, ELIZABETH A. LOUNDS and JAMES L. CONKLIN, Richmond, Virginia.

Blood transfusions have been recommended for the nutritional therapy of the sick or injured. The view that infused erythrocytes are a particularly good source of nutritive protein has been based on observations indicating little increase in nitrogen excretion following such infusions. Retention has been arbitrarily equated with utilization. Data contrary to this viewpoint have been obtained in patients in anabolic and catabolic nutritional states.

Patients with various types of serious injury or illness were maintained on constant oral dietary intakes. After suitable control periods, serologically identifiable erythrocytes were infused. Observations were made of body weight, nitrogen, sodium, and potassium balances, plasma and red cell volumes, erythrocyte survival, bone marrow morphology, and various liver functions. The apparent positive nitrogen balance immediately following the red cell infusions does not connote rapid metabolic utilization of the red cell protein. Rather, the rise in urinary nitrogen is slight because the red cell nitrogen participates directly in the body metabolic pool only over a period of many weeks. In no instance was the survival of the infused cells markedly shortened. A limited amount of nitrogenous compounds is made available indirectly through an associated depression of erythropoiesis. The decrease in red cell production is proportional to the quantity of red cells infused. Once the period of relative increase is over, erythropoiesis proceeds normally. The quantity of nitrogen which might be available as a result of depressed erythropoiesis even if complete is small, and only a similar relatively small quantity can be expected from the normal breakdown of infused cells. Erythrocytes, once anemia has been corrected, should not be infused for "feeding purposes"—the small contribution to the nutritional requirements of the patient does not warrant the associated risks.

The Relation of Stroke Output to Left Ventricular Filling Pressure in Normals and in Patients with Cardiovascular Disease. BENJAMIN N. LEWIS, FLORENCE W. HAYNES, HECTOR E. J. HOUSSAY and LEWIS DEXTER,* Boston, Mass.

Use of pulmonary "capillary" pressure as an index of left auricular pressure has made possible the study of left ventricular function in man. The relation of stroke output to filling pressure of the left ventricle has been studied at rest and during brief exercise in seven normal persons and in 16 individuals with hypertension, coronary disease, or lesions of the aortic valve. In four of seven normals there was slight increase of pulmonary "capillary" pressure on exercise with a concomitant increase of stroke output. In seven patients with mild manifestations of cardiovascular disease pulmonary "capillary" pressure was normal at rest, but increased above the normal range on exercise and this increase was all in cases associated with an increase of stroke output. In 8 of 9 individuals with moderate to severe signs and symptoms of congestive heart failure in whom pulmonary "capillary" pressure was abnormal at rest, further increases of pressure on exertion failed to produce any significant increase in stroke output. The increase of stroke volume resulting from increased diastolic fiber length of heart muscle and, therefore, from increased filling pressure (Starling's law) is only one of several mechanisms by which the normal heart can increase its minute output to meet increased bodily needs. This mechanism is, therefore, not demonstrable in all normal subjects. In patients with mild cardiovascular impairment, increase of filling pressure and therefore of diastolic size was apparently an important mechanism in increasing output during the stress of exercise and was demonstrable in all individuals studied. In those with more advanced symptoms in whom left ventricular filling pressure was already abnormal at rest, increased diastolic pressure was already optimal or nearly so for these diseased hearts, since further increases of filling pressure on exercise produced no increase in stroke output.

Reflex Vagal Action on the Heart in Man Produced by Ischemic Stimulation of the Carotid Body. ARTHUR J. LINENTHAL, Boston, Mass. (Introduced by Herrman L. Blumgart).

Anoxic *chemoreceptor* stimulation, of recognized importance in the reflex control of respiration, has not heretofore been described as a cause of reflex vagal effects on the heart in man. We have found that constant, continued pressure over the carotid artery bifurcation, in normal subjects as well as in cardiac patients, produces striking vagal action on the heart and marked respiratory stimulation, with or without a preceding carotid sinus *pressoreceptor* cardiac reflex. Changes in consecutive cycle lengths, the most sensitive cardiac vagal effect, were measured from electrocardiograms; changes in respiratory rate and volume were determined from pneumograms. These cardiac and respiratory reflexes are attributed to ischemic (anoxic) stimulation of the carotid body.

The carotid *body* cardiac reflex is of vagal origin:

cycle length increases; respiratory sinus arrhythmia appears or is exaggerated, with particular prolongation of the expiratory cardiac cycle; atropine blocks these effects without influencing the respiratory changes. This reflex action appears after 20 to 40 seconds of pressure, becomes maximal in about 60 seconds, and may then show slow, cyclic variations in intensity. The simultaneous increase in respiratory rate and volume is not the cause of the carotid body cardiac effect: similar voluntary respiratory changes produce decreased rather than increased cycle lengths. The vagal cardiac effect of continued carotid *sinus* stimulation, on the other hand, is maximal in the first few seconds of pressure, returns to the control level after 10 to 20 seconds, and is unaccompanied by respiratory changes.

Stimulation of chemoreceptors in the carotid body and elsewhere may contribute to the development of the frequent and often serious disturbances of cardiac rate and rhythm which occur during conditions of systemic anoxia. The hitherto unexplained association of cardio-vagal and respiratory stimulation during Cheyne-Stokes hyperpnea may also involve this reflex mechanism.

The Role of Carbon Dioxide Tension in the Pathogenesis of Pulmonary Hypertension. FRANK W. LOVEJOY, JR., PAUL N. G. YU, R. E. NYE, JR. and H. A. JOOS, Rochester, N. Y. (Introduced by W. S. McCann).

A statistical analysis of the relationship of mean pulmonary arterial pressure to possible determinants has been made in 46 patients, 33 of whom had chronic pulmonary disease. Patients with mitral stenosis or congenital heart disease were excluded. A significant relationship was found between pulmonary arterial pressure and (a) the P_{CO_2} of mixed venous blood, (b) P_{CO_2} of arterial blood, (c) the degree of emphysema as expressed in terms of per cent residual air volume (% of the total capacity), and (d) the degree of oxygen saturation of arterial blood.

The hemodynamic effects of CO_2 added to inspired air during cardiac catheterization have been studied in a group of 30 patients. Complete data were available in 11 patients breathing 3.6% CO_2 and in ten patients breathing 6% CO_2. Analysis of these cases revealed that a rise in mean pulmonary artery pressure and pulmonary arterial resistance occurred in both groups, but was proportionately greater in the group breathing 6% CO_2. Neither anoxia nor increased blood flow was responsible for these changes. In general, the patients with most emphysema showed the greatest rise in pulmonary arterial pressure. Two normal subjects showed a fall in pulmonary arterial pressure when breathing 6% CO_2. The implication of these findings is discussed.

Effects of Alterations of Body Potassium on Digitalis Toxicity. BERNARD LOWN, JOHN M. WELLER, NORMAN WYATT, ROLF HOIGNE and JOHN P. MERRILL, Boston, Mass. (Introduced by Samuel A. Levine).

Utilizing acetyl strophanthidin, an ultra-rapid digitalis-like body, and the Kolff-type of artificial kidney, a method has been evolved for studying the effects of selective electrolyte alterations on the action of digitalis in the intact animal.

The relation of body potassium to the toxic action of digitalis has been explored in seven dogs. Control digitalizations given every two hours without evidence of cumulation were carried out prior to dialysis and during dialysis against a bath with a potassium of 4mEq/L. Similar digitalizations were carried out during dialysis against baths containing zero and 8mEq/L potassium. The toxic end points for digitalization were ventricular premature beats or ventricular tachycardia.

Potassium extraction during two hours dialysis averaged 26mEq. per animal, lowered serum potassium to 2.3mEq/L and resulted in: 1) A 60% decrease in dosage of acetyl strophanthidin necessary for toxicity. 2) A five to tenfold increase in duration of toxicity. 3) Toxicity consisting of bigeminal rhythms or alternative bidirectional tachycardias not observed without potassium depletion. Continued potassium extraction while the serum level was maintained constant resulted in accentuation of the above changes.

Potassium addition raising the serum level to an average of 6.5mEq/L resulted in: 1) A 50% increase in dosage of acetyl strophanthidin for toxicity. 2) Toxicity characterized by marked transiency of ventricular irritability with suppression of ventricular but not auricular manifestations of digitalis overdosage.

Determination of Estrogen Excretion During Chorionic Gonadotrophin Therapy as a Test of Human Leydig Cell Function. WILLIAM O. MADDOCK, Detroit, Michigan, and WARREN O. NELSON, Iowa City, Iowa. (Introduced by Gordon B. Myers).

Biological assay of urinary estrogens has proved a reliable and sensitive measure of Leydig cell response to chorionic gonadotrophin as indicated by the following observations. The administration of 5,000 units of chorionic gonadotrophin three times weekly for six or more weeks produced a five- to twenty-fold increase of urinary estrogens in each of seven men having functioning testes, but no increase in three men lacking testes. Urinary estrogens rose within twenty-four hours after initiating therapy and remained elevated throughout treatment. It was concluded that estrogens originated from Leydig cells since repeat testicular biopsies obtained from four patients during therapy revealed a marked increase of Leydig cells but regressive changes in seminiferous tubules. In contrast to the striking and sustained elevation of estrogen excretion, the rise in urinary 17-ketosteroids was less marked (approximately two-fold) and was more variable, individual values during therapy often falling within the pretreatment range.

In each of three hypogonadotrophic eunuchoids and in three of five men having adult hypopituitarism, initially low estrogen levels increased markedly during chorionic gonadotrophin therapy. Pretreatment testicular biopsies from the six responsive patients revealed inactive structures which appeared capable of being stimulated. The

two unresponsive patients had completely hyalinized testes. Biopsies obtained from two of the eunuchoids after three months of therapy revealed growth and development of Leydig cells.

Determination of urinary estrogens before and following a standard dose of chorionic gonadotrophin is therefore suggested as a test of Leydig cell function, analogous to the test of adrenal cortical function in which 17-ketosteroid determinations are used to evaluate response to ACTH.

The Effect of Body Position on Regional Alveolar Air Concentrations. C. J. MARTIN, F. CLINE, JR. and H. MARSHALL, Seattle, Washington. (Introduced by R. H. Williams).

Unequal ventilation of the lungs has been measured at mouth level by various techniques. We have measured end-expiratory alveolar oxygen and carbon dioxide concentrations from upper and lower lobe bronchi in man and determined the effect of body position upon these concentrations. Twenty-one normal volunteers under basal conditions were given phenobarbital (0.1 gm.) and atropine (0.41 mg.) as premedication. The pharynx and larynx were anesthetized with cocaine (5%) in epinephrine (1:4000). Radio-opaque plastic (2 mm. O.D.) catheters were guided into the trachea, and under fluoroscopy into the desired lobar bronchus. Simultaneous end-expiratory gas samples were obtained from the right upper and right lower lobe bronchi with the subject in the supine, upright and Trendelenburg positions. Duplicate gas analysis was conducted in a Scholander 0.5 cc gas analyzer. After all samples were obtained a small amount of contrast mixture was instilled through each catheter and films taken.

In the supine position the right upper lobe alveolar oxygen values are below those of the right lower lobe by approximately one volume per cent. The difference is not statistically significant ($p = 0.02$). In the upright position the right upper lobe oxygen values are above lower lobe values by approximately two volumes per cent. The difference is statistically significant ($p = 0.001$). The direction of change in carbon dioxide values is opposite to that of oxygen. In the Trendelenburg position no significant difference has been found between simultaneous upper and lower lobe samples.

Lobar differences in alveolar gas concentrations exist. Particularly significant is the higher oxygen concentration in the upper lobe as compared with lower lobe when the subject is upright. This difference is of interest in relation to current theories explaining apical localization of adult pulmonary tuberculosis. The higher oxygen values in the upper lobe when upright would seem to favor growth of the tubercle bacillus.

The Mechanism of Protoveratrine-Induced Oliguria. EDWARD MEILMAN, Boston, Mass. (Introduced by Joseph E. F. Riseman).

The lowered blood pressure induced by intravenously administered protoveratrine in persons with hypertension is attended by a marked diminution of urine volume. The oliguria is accompanied by a striking diminution of sodium and chloride excretion which differentiates the process from oliguria due to anti-diuretic hormone. Potassium excretion during this time diminishes but slightly. That active absorption of water from the glomerular filtrate is involved is indicated by the marked increase in concentration of inulin or paraaminohippurate. Although the hypotension may cause acutely a significant decrease in glomerular filtration rate and in effective renal plasma flow, these may return to control values without increasing the excretion of sodium or the volume of urine. The duration of this effect is about four hours. Thereafter, despite a continued low level of blood pressure, urine volume and sodium excretion rise. Continuous hydration causes an increase in urine volume before this time, but the diminution in sodium excretion is not affected.

Forty to sixty minutes after intravenous injection of a mercurial-theophylline complex (mercuhydrin) during the period of oliguria, a very marked rise in the output of both sodium and water results.

These observations indicate that either the acute hypotension, or the acute fall in GFR or ERPF, initiates a marked tubular absorption of sodium and chloride, but not potassium, and that water is concurrently osmotically resorbed. This process persists despite recovery of both GFR and ERPF to control values. The fact that mercury overcomes this effect suggests that this resorption of sodium may be mediated by a tubular sulfhydryl enzyme system.

Occurrence of Hepatitis in Cortisone-Treated Rats Following Inoculation with Human Infectious Hepatitis Serum. GEORGE W. MELCHER, JR., J. W. BLUNT and CHARLES RAGAN,* New York, N. Y.

Rats made hyperadrenal by the administration of cortisone acetate were inoculated subcutaneously with human serum obtained from an individual with viral hepatitis. This serum (obtained from Dr. Donal R. Fitch) had previously been tested on human volunteers and had been proven capable of producing the clinical picture of infectious hepatitis.

Diffuse focal hepatitis was observed microscopically in the livers of these rats. Subsequent inoculation of a suspension prepared from the livers of these animals produced hepatitis in a second group of cortisone-treated rats.

No hepatitis has been observed in the following groups of control animals: (a) normal rats given the same hepatitis serum or a liver suspension prepared from the rats showing liver lesions, (b) hyperadrenal rats given normal human serum or a suspension of normal rat liver.

Decreased Circulation in the Great Toe as the Earliest Manifestation of Vascular Disease in Diabetes Mellitus. MILTON MENDLOWITZ,* EDWARD B. GROSSMAN and SAMUEL ALPERT, New York, N. Y.

The development of vascular disease in a group of 40 young males with diabetes mellitus was investigated by

the measurement of blood flow in the great toe calorimetrically after release of sympathetic nerve tone by indirect heating supplemented by the intravenous administration of 5 mg/Kg of tetraethylammonium chloride. All of these patients had palpable pedal pulses, normal oscillometric tests and no overt evidence of renal or cardiac impairment. When the occasional cases with calcification of the arteries as demonstrated by roentgenograms of the legs or with retinal changes were excluded, it was found that approximately one-fourth of the remaining cases revealed blood flow values below the lower limit of the normal range on repeated testing. Statistical evaluation of the difference between the diabetic and a normal control group consisting of 30 patients also repeatedly tested revealed a significance ratio greater than 3.0. No correlation could be established between the decreased hallucal circulation and the cholesterol-phospholipid ratio. There was also no significant correlation between the apparent duration, severity or adequacy of control of the diabetes and the circulatory changes. None of these patients had known diabetes for more than ten years or was under the type of control which could be classified as excellent.

Renal Damage in Rats Fed Large Quantities of Sodium Chloride. GEORGE R. MENEELY, ROBERT G. TUCKER, STEWART H. AUERBACH and WILLIAM J. DARBY,* Nashville, Tennessee.

Six groups of thirty male Sprague-Dawley rats five weeks old were placed on purified diets complete and identical except for sodium chloride. The control group received 0.15% sodium chloride in the diet. The five experimental groups received progressively higher proportions of sodium chloride: 2.8, 5.6, 7.0, 8.4 and 9.8%, respectively. The diets and demineralized water were available ad libitum. When first placed on the experimental diets, marked polydipsia and polyuria occurred. After three days these rats appeared to be severely dehydrated. Two weeks later the rats all looked healthy and were gaining weight regularly.

During the third and fourth months edema developed in 9% of the rats eating the three highest levels of sodium chloride. Sudden weight gains of 40 to 160 grams occurred. Extracellular water measured with radioactive sodium rose from the control level of 25% to 57% of body weight. These edematous animals were severely anemic and showed lipemia and hypoproteinemia. At autopsy there were obvious edema and anemia. Otherwise, microscopically, all the tissues except the kidneys appeared normal. In relatively bloodless glomerular tufts there were foam cells containing large amounts of fat staining material. The tubules exhibited a nephrotic degeneration and contained also much fat staining material. There were lesser deposits of lipoid in some of the arterioles.

Three edematous animals survived this phase, rapidly lost their extra fluid and became extremely emaciated and cachectic. Again, all tissues seemed essentially normal except for a renal lesion similar to that described above but more extensive. Many glomerular tufts were obliterated. Tubular degeneration was extreme. No evidence of adrenal failure was found.

Psychological and Somatic Factors in Coronary Artery Disease. HENRY H. W. MILES, SAMUEL WALDFOGEL and EDNA L. BARRABEE, Boston Massachusetts. (Introduced by Stanley Cobb).

Psychiatric interviews, a detailed social inventory and a battery of psychological tests were carried out upon a group of 45 men with uncomplicated coronary artery disease. Forty-eight healthy control subjects, 20 of whom were carefully matched with 20 of the coronary patients with respect to age, somatotype, ethnic origin and socioeconomic background, were similarly studied. All subjects had been previously investigated with great care so that data concerning past medical history, anthropological measurements and blood chemistry were available.

After consolidating the information gathered by various means, we summarized for each individual such factors as family relationships, characteristic patterns of reaction to the stresses of life, characteristic personality traits and ego defenses and (in the 45 coronary patients) the specific setting in which the illness became manifest.

The two groups were compared in terms of personality factors and also in terms of other factors such as family incidence of cardiovascular disease, history of physical stress and strain, somatotype and blood lipid levels.

The discussion is developed in the frame of reference of the concept of multiple causality. Those characteristics of the individual as well as those factors of the environment which seem to be relevant and causal are considered, with some tentative interpretations of the correlations found by us and by other investigators.

Fructose Metabolism in Diabetic Subjects. MAX MILLER,* JAMES W. CRAIG, HIRAM WOODWARD, JR., J. EVAN OWENS and WILLIAM R. DRUCKER, Cleveland, Ohio.

Previous work from this laboratory has shown that the intravenous administration of fructose to diabetic subjects in the absence of exogenous insulin produced the same blood pyruvic acid rise as in normal subjects, while glucose produced a slight or delayed rise. The blood fructose disappearance curve and the amount of urinary fructose were the same as in normals. Serum inorganic phosphorus fell after fructose but not after glucose. Hepatic vein catheterization studies showed that the splanchic uptake of fructose was similar in diabetic and normal subjects and was greater than the uptake of glucose.

Others have stated that the apparent advantage of fructose over glucose in the diabetic was lost if the oral administration of fructose was continued for several days. Two diabetic patients were given 50 and 100 grams of fructose, respectively, each morning without insulin. No increase in fructose excretin or change in the fructose tolerance curve was noted during 9 days of either oral or intravenous administration. At the end of the periods fructose still retained its advantage over glucose, as

measured also by total hexose excretion, rise in blood pyruvate and fall in phosphorus.

Two cases of diabetic acidosis have been treated with fructose, saline and insulin and the results compared with cases in 2 previous series treated with glucose, saline and insulin, and with saline and insulin alone. The administration of 175 grams of fructose during the initial 6 hours resulted in the excretion of only 7 and 3 grams, respectively, and blood fructose levels did not exceed 100 mg.%. Blood glucose levels fell approximately at the same rate as in the series treated with saline and insulin alone. Falls in blood ketone levels, water balance data, and changes in blood pyruvate in the 3 series will be discussed.

Phospholipid Turnover in Man. HUGO W. MOSER, Boston, Mass. (Introduced by Kendall Emerson, Jr.).

A method has been developed which makes it possible to calculate the per cent of plasma phospholipid turned over per unit time. Following the intravenous injection of a tracer dose of carrier-free inorganic P_{32}, the specific activity of plasma lipid and urinary phosphorus is followed for 96 to 120 hours. We have confirmed Handler's findings that the specific activity of urine phosphorus is almost identical with that of plasma inorganic phosphate. When urine phosphate is plotted against time it can be expressed as the sum of two exponentials.

The data obtained indicate that the inorganic phosphate of the urine is a closely approximate measure of the immediate precursor of lipid phosphorus, according to the criteria of Zilversmit. Knowing the simultaneous rates of change of the specific activity of lipid phosphorus and its immediate precursor an equation can be derived for the calculation of the actual percentage turnover of plasma phospholipid per unit time. Since Chaikoff has shown that plasma and liver phospholipid are in equilibrium and form a single pool, we are presumably measuring the turnover rate in this pool. In three normal subjects from 1.4 to 1.9 per cent of phospholipids were turned over per hour, whereas in three nephrotics this value was between 0.6 and 0.8 per cent. In a patient with myxedema 0.6 per cent was turned over per hour before treatment and 1.1 per cent during therapy with thyroid.

In these patients the plasma phospholipids were elevated to the same extent that their phospholipid turnover rate was depressed, as compared to the normal subjects. The absolute quantity of plasma phospholipid renewed per unit time, therefore, was the same in the normal and pathological subjects.

Preliminary Studies on the Purine and Pyrimidine Bases of Human Bone Marrow as Determined by Paper Chromatography. I. Variations in Pernicious Anemia in Response to Therapy. JOHN F. MUELLER, HELEN STIX GLAZER and RICHARD W. VILTER,* Cincinnati, Ohio.

Numerous biologic studies have linked folic acid and vitamin B_{12} to the formation of pyrimidines and purines. Responses of patients with pernicious anemia to thymine and to a lesser extent to uracil suggest a similar relationship. Since thymine is present only in desoxyribonucleic acid, an abnormality in this substance might be expected; however, cytochemical and spectrophotofluorometric techniques have suggested some defect in ribonucleic acid metabolism. It seemed advisable, therefore, to measure the qualitative and quantitative changes in the purine and pyrimidine bases of the bone marrow and apply this method to a study of pernicious anemia before and after specific therapy.

The buffy coat from a bone marrow aspirate is treated with cold trichloracetic acid. Lipids are extracted with hot ether-alcohol and the residue hydrolyzed with perchloric acid. The purines and pyrimidines are separated by paper chromatography with butyl alcohol as solvent. An alkaline pH separates guanine, cytosine, thymine and a mixture of adenine-uracil. An acid medium allows separation of thymine and uracil. The spots containing the various bases are detected in ultraviolet light, photographed, cut out and eluted in weak hydrochloric acid. Amounts in the eluate are determined in the Beckman spectrophotometer and expressed as molar ratios.

Bone marrows from normal persons have been studied and have contained all the known bases. Uracil is present in small amounts. In three of four patients with pernicious anemia in relapse studied so far the following observations have been made. (1) Thymine is low and gradually increases after therapy when determined on alkaline strips. (2) The ratio of uracil to thymine determined on acid strips decreases ten-fold after therapy suggesting that thymine increases as uracil decreases. These studies point to a fundamental defect in pyrimidines in pernicious anemia which is corrected by folic acid and vitamin B_{12}.

The Effects of Cortisone and ACTH on Splanchnic Circulation and Metabolism. J. D. MYERS* and W. JAPE TAYLOR, Durham, North Caroline.

The profound metabolic effects of cortisone and ACTH might be expected to be associated with alterations in hepatic circulation and splanchnic metabolism of oxygen and glucose. The hepatic blood flow (estimated by hepatic venous catheterization and the bromsulphalein tecnic of Bradley) and splanchnic balances of oxygen and glucose have been measured in a) 8 human subjects, with only minor ailments, who received 200 mg. of cortisone orally approximately four hours before study, and b) 12 persons with illnesses which required ACTH. Each patient on ACTH served as his own control, the observations made after one day to three weeks on ACTH being compared to control studies done before or after the period of treatment. No pre- or post-treatment control data were collected on the cortisone patients; their findings are compared with data from 62 similar but untreated persons.

Cortisone resulted in a mean increase in hepatic blood flow of 43 per cent ($p < 0.01$). Splanchnic oxygen consumption was augmented by 26 per cent ($p < 0.01$) hepatic arterio-venous oxygen difference decreasing only slightly and insignificantly. Cortisone produced no measurable

alterations in arterial blood glucose concentration, hepatic A-V glucose difference, or splanchnic glucose production.

The effects of ACTH on hepatic blood flow and splanchnic oxygen consumption depended upon the control levels of those factors. Five of the 12 subjects had elevated control flows and oxygen consumptions presumably due to their diseases, and ACTH resulted in no further augmentation. The other 7 subjects, with normal control values, showed a mean increase in flow of 28 per cent ($p < 0.01$) and in oxygen consumption of 26 per cent ($p < 0.05$). Again hepatic A-V oxygen difference was unchanged. Three of the subjects on ACTH acquired hyperglycemia and several showed rises in splanchnic glucose production; the two phenomena were not related, however. Neither drug affected BSP clearance.

In summary, both cortisone and ACTH appear to raise hepatic blood flow and splanchnic oxygen consumption above the usual basal levels.

Demonstration of Existence of Long Term "Asymptomatic" Blood Carriers of Hepatitis Virus with Confirmation in Human Volunteers: Relationship of Carriers to Acute Viral Hepatitis in Whole Blood Recipients. JOHN R. NEEFE,* ROBERT F. NORRIS, JOHN G. REINHOLD, JOHN W. OLIPHANT, DONALD R. FITCH, RICHARD K. WATANABE, CHARLES B. MITCHELL and DAVID S. HOWELL, Philadelphia, Pa.

During the past 18 months, 15 patients with acute viral hepatitis, presumably acquired from whole blood transfusions, have been observed. Nine of the patients had had only a single whole blood transfusion, four had 2 or 3 transfusions each and the remaining three had multiple transfusions. All of the donors involved with 12 of the cases became available for careful study which included complete history, physical examination, and a comprehensive group of hepatic tests. Needle biopsies of the liver were obtained on 3 of the donors. Serum specimens for transmission experiments in human volunteers were obtained at intervals from the suspected donors. None of the donors had histories of jaundice or hepatitis and all met the usual criteria required by most large blood collecting centers.

Definite evidence of hepatic disturbance (histologically confirmed in 3) was demonstrated in the blood donors for 10 of the 12 patients with acute viral hepatitis. Serum specimens obtained from 5 of the suspected donors three to twelve months after the time of their original infectious donations subsequently were tested for infectivity in human volunteers. The development of hepatitis with jaundice in 4 of the 5 groups of volunteers inoculated with the 5 respective sera demonstrated conclusively: (1) That the suspected donors were the sources of infection of the original patient recipients and (2) that 4 of the 5 donors were "asymptomatic" blood carriers of hepatitis virus over a minimum period of 3 to 12 months.

The results appear to be of considerable significance in relation to the serious problem of blood transmitted hepatitis. Demonstration of the existence of "asymptomatic" blood carriers represents confirmation of a factor of major epidemiological importance, previously suspected but not proved. The findings to date suggest routine "hepatic" testing of donor blood might provide one additional means of decreasing the present serious incidence of blood transmitted viral hepatitis.

The Direct Measurement of the Extracellular Phase of Tissues. G. NICHOLS, JR., N. NICHOLS, W. B. WEIL and W. M. WALLACE,* Boston, Massachusetts.

Measurements of the extracellular fluid in the human subject by inulin and thiosulfate give volume distributions so small that they are difficult to reconcile, biochemically or morphologically, with balance data and tissue analysis in experimental animals and man. To clarify this problem, the distribution of a single injection of inulin and thiosulfate was measured directly in the tissues of eight nephrectomized dogs, at varying time intervals, and compared with simultaneous electrolyte, water and collagen analyses of these tissues.

In all the tissues studied, the chloride space exceeded the thiosulfate space, and this exceeded the inulin space. Dense connective tissue, such as tendon, showed an increase in size of the thiosulfate and inulin spaces from 3 to 6 hours. At 6 hours thiosulfate space represented 75% of the extracellular phase; inulin, 25%. Thiosulfate space was 60% of chloride space in muscle, 75% in skin, and showed no increase from 3 to 6 hours. Inulin spaces in muscle and skin increased slightly from 3 to 6 hours, reaching 50% of the chloride space.

Since neither thiosulfate or inulin spaces reach the size of the plasma ultrafiltrate phase of tendon or skin in 6 hours, they are not accurate measures of the extracellular fluid within this time interval. However, from a morphologic point of view, inulin or thiosulfate space in tissues, plus that fraction of the connective tissue phase which remains unpenetrated at any given time, defines a space which is only slightly smaller than the space defined by chloride, suggesting that when chloride space is corrected for the higher concentration of chloride in connective tissue, it is both biochemically and morphologically the best measure of the extracellular space.

Hypertension and Pyridoxine Deficiency in the Rat. NORMAN S. OLSEN and WILLIAM E. MARTINDALE, Nashville, Tennessee. (Introduced by John B. Youmans).

Recent experimental observations indicate that certain types of hypertension may be of dietary origin. In the present report we have investigated the relation of elevated blood pressure to the vitamin B_6 group of compounds, which is known to be important in the metabolism of amino acids.

Pyridoxine deficiency was produced in young Wistar rats by means of a deficient diet supplemented by desoxypyridoxine. In addition to the typical clinical symptoms these animals exhibited an increase in blood pressure highly significant statistically as compared to litter-mate controls. A group of the hypertensive-deficient rats was placed on an adequate diet supplemented with pyridoxal.

A rapid increase in weight together with a significant fall in blood pressure to normal levels was noted. These observations were duplicated in older animals.

Further studies on organ weights, oxygen uptake of tissue homogenates, vitamin B_6 assay of tissues and histological survey of tissues were carried out. There was a significant increase in the adrenal weights of the deficient animals which was paralleled by a hypertrophic trend in the ventricular mass. The oxygen uptake of kidney and liver homogenates from the deficient animals was found to be about 30 per cent less than in the control group. Vitamin B_6 assays on the same tissues showed changes in the same direction. The histological survey gave the general impression of no arterial or arteriolar disease and no significant irreversible disease process.

Mechanisms of Adjustment in the Cerebral Circulation Upon Assumption of the Upright Position. JOHN L. PATTERSON, JR., and JAMES V. WARREN,* Atlanta, Georgia.

Both the blood flow through the human brain and the arterial pressure at brain level fall when the upright position is assumed, but the percentile diminution in blood flow is considerably smaller.

Continuous oximetric determinations of changes in the cerebral arteriovenous oxygen difference were made during 65° (head-up) tilting. Since the oxygen consumption remains stable in non-fainting subjects, the reciprocal of the arteriovenous oxygen difference will vary directly with the blood flow. Arterial, internal jugular and cerebrospinal fluid pressures were measured with strain gauges mounted at ear level.

In this series mean arterial pressure was 19.2 mm. Hg (24.3%) lower, and the venous pressure 7.3 mm. Hg lower in the upright than in the recumbent position. Internal jugular pressure actually fell to slightly subatmospheric levels in most subjects. The value of $1/(A\text{-}V)O_2$ averaged 14% less in the upright position. Changes in cerebrospinal fluid pressure closely paralleled the changes in jugular pressure.

The percentile fall in net perfusion pressure (arterial minus internal jugular pressure) was actually only slightly greater than that in the cerebral blood flow, owing to the fall in venous pressure.

The changes in cerebrospinal fluid pressure probably counterbalanced the fall in intracranial venous pressure and capillary pressure and thus prevented compression of these vessels.

Because a siphon effect appeared possible, experiments with a brain model were done, using thin-walled rubber tubing to simulate the jugular vein. These indicated that collapsible tubes can function as the descending limb of a siphon. Potential vasodilator stimuli were present in the form of increased capillary CO_2 tension and decreased O_2 tension resulting from reduction in CBF with maintenance of normal metabolism.

These results do not require the assumption of any cerebral pressoreceptor-vasomotor mechanism, and suggest operation of the siphon principle in this and possibly other areas of the circulation.

Physiological Effects of Bilateral Adrenalectomy in Man. O. H. PEARSON,* V. P. HOLLANDER, C. D. WEST, W. F. WHITMORE and H. T. RANDALL, New York, N. Y.

Bilateral adrenalectomy has been carried out in seventeen patients with advanced cancer during the past year. It has been possible to maintain these patients satisfactorily during and after surgery with cortisone as the only replacement therapy. Patients maintained for many months on oral cortisone (average dose 50 mgs daily) failed to manifest symptoms or signs of adrenal insufficiency or of hypercorticism. Among the group of patients maintained on approximately 50 milligrams of cortisone daily, we have observed normal eosinophile counts, normal values for urinary 17-ketosteroids and formaldehydogenic steroids, growth of pubic hair, normal insulin tolerance, normal water tolerance, normal glucose tolerance, normal blood sugar after prolonged fasting, reduction of urinary sodium excretion on salt deprivation, and the ability to withstand moderate stress.

Abrupt withdrawal of oral cortisone was associated with the characteristic symptomatology of adrenal insufficiency which began within twenty-four hours and intensified so that by the fifth to eighth day the patient's status was critical. Alterations in water and electrolyte balance were characterized by prompt antidiuresis, hemodilution, hyponatremia and hypochloremia. A negative balance of sodium and chloride usually occurred but was not associated with loss in body weight, presumably due to water antidiuresis. Potassium retention and hyperkalemia did not occur. Restitution of cortisone therapy resulted in profound water diuresis and prompt clinical improvement. Sodium and chloride were retained and the serum levels of these electrolytes returned to normal.

The clinical and physiological changes which occurred on cortisone withdrawal from the adrenalectomized patient have been closely simulated by the administration of pitressin tannate while cortisone therapy was maintained.

These observations indicate that water intoxication is an important feature of the physiologic effects of adrenal insufficiency in man.

A Syndrome Resembling Impending Hepatic Coma Produced in Patients with Cirrhosis of the Liver by the Administration of Certain Nitrogenous Substances. GERALD B. PHILLIPS, ROBERT SCHWARTZ, GEORGE J. GABUZDA, JR. and CHARLES S. DAVIDSON,* Boston, Mass.

A syndrome indistinguishable from impending hepatic coma and typically consisting of mental disturbances and a characteristic tremor and electroencephalographic pattern was produced in five of nine chronic alcoholics with advanced hepatic cirrhosis by the oral administration of large quantities of certain nitrogenous substances. The abnormal signs always reversed within one or two days after cessation of the administration of these substances, which were given during periods of from two to twenty-two days.

Two patients developed the syndrome when given an

ammonium containing cation exchange resin and again when given ammonium chloride. The characteristic signs did not recur in one of these two patients during a comparable degree of acidosis induced by calcium chloride administration. A similar reaction was observed in a third patient during one course of di-ammonium citrate and three courses of urea, but not during a period of high dietary protein intake. However, the syndrome did appear in two other patients following an increase in dietary protein intake. Four other patients failed to show any untoward effect when given separate courses of one or more of these substances, including increased dietary protein.

Serial determinations of serum pH, carbon-dioxide, sodium and potassium, and of blood nonprotein nitrogen failed to reveal alterations which correlated with the clinical changes. The blood ammonia content (diffusible alkali measured by the Conway microdiffusion technique) was often increased during clinical reactions, but the changes were not consistent.

Conclusions: A syndrome apparently identical with impending hepatic coma was produced in five patients with advanced hepatic cirrhosis by the oral administration of an ammonium containing cation exchange resin, ammonium chloride, diammonium citrate, urea, and large quantities of dietary protein.

The administration of these substances to patients with liver disease may by hazardous.

Effect of Thiocyanates on Sodium Excretion in Normotensives and Hypertensives. KERMIT L. PINES and GEORGE A. PERERA,* New York, N. Y.

A depressor action of thiocyanates in essential hypertension has been described by many investigators, although the percentage of responsive patients has varied considerably in different clinics. Because of apparent relationships between electrolyte metabolism and arterial tension, balance studies were undertaken before and during thiocyanate administration in 21 normal subjects and 14 patients with uncomplicated essential hypertension.

Following at least a six-day control period on a constant dietary regimen, sodium or potassium thiocyanate was given by mouth in divided doses daily for four days with resultant peak serum levels in the 4-7 mgs. per 100 cc. range. The sodium content of the diet was varied widely in different individuals in both groups, and observations were made under both ambulatory and resting conditions. The average urinary sodium excretion per day for the final four days of the control period was compared to that during the four days on thiocyanate, appropriate corrections being made when the sodium salt was employed.

Following treatment, the average daily urine sodium rose 12 meq. (15 per cent) in the entire normotensive series, and 27 meq. (33 per cent) in the hypertensive group. Among the 21 normotensives were five subjects in whom mean urinary sodium levels during thiocyanate administration exceeded the dietary intake; in contrast, all the hypertensives lost sodium in greater amounts than that contained in their diet.

In view of the effects of rigid salt restriction in hypertensive disease, the possibility is raised that thiocyanates may exert a depressor effect through similar mechanisms. Furthermore, this study provides added evidence for a difference in metabolic response of hypertensives when compared to normals.

Clinical and Experimental Studies with Tritium. T. C. PRENTICE, W. SIRI, N. I. BERLIN and R. J. PARSONS, Berkeley, California. (Introduced by John H. Lawrence).

The present study was undertaken to develop further the use of tritium (H^3) for routine body water measurement and to extend the investigation of total body water to a variety of disease states. It was necessary, in conjunction with the above, to evaluate several factors which could influence the accuracy of total body water determinations with tritium-labeled water. These were: (1) the time required for complete mixing throughout body water following intravenous administration of tritium-labeled water; (2) the possible existence of a significant isotope effect due to the large mass increment of tritium; and (3) the magnitude of the error introduced by exchange of tritium with the solid constituents of the body.

Total body water determinations were carried out in 57 patients, 20 of whom were normal, the remaining 37 being patients with cardiac, liver, and blood abnormalities.

Mixing appeared to be "complete" in all patients within three hours. Comparison of total body water as measured simultaneously with tritium and antipyrine in 15 patients revealed the former to be consistently 2% of body weight greater than the latter. These results compared very closely with deuterium-antipyrine ratios found previously, thus making any tritium isotope effect unlikely. This 2% difference could be accounted for quantitatively by exchange of tritium with solid constituents, as measured in a group of rabbits.

No characteristic patterns of total body water were found in any given clinical group. It was concluded that wide variations in individual fat content were responsible for the surprising over-all uniformity in proportion of body water in subjects with and without abnormal accumulation of fluid.

The Wolff-Parkinson-White Syndrome. MYRON PRINZMETAL,* REXFORD KENNAMER, ELIOT CORDAY, JOHN A. OSBORNE, JOSHUA FIELDS and L. ALLEN SMITH, Los Angeles, Calif.

Various types of the Wolff-Parkinson-White (WPW) aberration were produced experimentally in 25 dogs by applying a direct electrical current of subthreshold intensity to the A-V node. Stimulation of the same region of the A-V node, at different rates, resulted in several forms of supraventricular arrhythmias, which were identical lesion.

A new technique of high-speed cinematography has enabled the cardiac movements to be recorded simultane-

ously with the electrocardiogram. By this means it was observed that a premature localized contraction in one ventricle was related to the premature portion of the R wave.

In addition to the usual cogenital type, numerous examples of an acquired nodal type of WPW aberration have been observed clinically in patients with organic heart lesions involving the region of the A-V node. The most common cause seems to be posterior myocardial infarction.

The hearts of three such patients with acquired nodal type of WPW syndrome were examined histologically. In two, myocardial infarction and fibrosis had involved the A-V node, while in the third this region was invaded by a fibrocalcific lesion.

Experimentally, section of the bundle of His always prevented the production of WPW aberration. Therefore, the impulse must reach the ventricles through the normal conduction system. It is unnecessary to presume that abnormal conduction pathways, such as the bundle of Kent, are responsible.

Newcastle Disease in Man. ROBERT W. QUINN, ROBERT P. HANSON, JOHN W. BROWN,* CARL A. BRANDLY, Madison, Wisconsin.

Interest in Animal-Human disease relationships has been stimulated at the University of Wisconsin, by collaboration of departments in the Medical School and College of Agriculture. The occurrence in man of Newcastle Disease of Fowls has received attention recently. This report is concerned with the observations obtained during the course of three cases of infections which occurred in laboratory workers. Two individuals were accidentally exposed to Newcastle Disease virus by aerosol spray; one of these also accidentally punctured his skin with a needle contaminated with active virus. The third patient had washed contaminated glassware. Symptoms appeared in approximately 24 hours. The most prominent and constant clinical features were malaise, photophobia with conjunctivitis, sore throat with superficial ulcerations of buccal mucosa, and preauricular adenitis. The duration was about 7 days, the illness of 1 of the 3 individuals was very mild, almost inapparent.

Virus isolations were performed by inoculation of embryonated eggs, followed by hemagglutination-inhibition tests on allantoic fluids of infected embryos. Newcastle Disease virus was obtained from conjunctival sac washings, 2 cases, nasal secretions, 1, throat washings, 2, salivia, 1, blood, 1, and urine, 1.

Newcastle Disease appeared in chickens in the United States in 1941, and spread rapidly to become a major disease problem of poultry in this country. Thirty-three cases of the disease in humans were reported through 1950. Newcastle Disease virus was isolated in 7; viremia was demonstrated in 1. The observations of investigators in Minnesota during a recent outbreak in a poultry eviscerating plant will add information.

A notworthy feature of the present report is the isolation of Newcastle Disease virus from one patient with nearly inapparent infection.

Metabolic Effects of a New Growth Hormone Preparation. M. S. RABEN, V. W. WESTERMEYER and A. LEAF, Boston, Massachusetts. (Introduced by E. B. Astwood).

Growth hormone was recovered and concentrated from the residual solution following the purification of corticotropin with oxycellulose. Glacial acetic acid extraction at 70° C. and subsequent treatment in acid environment established conditions markedly different from those generally employed in the preparation of growth hormone. This concentrate was equal in growth-promoting activity to those of Wilhelmi and of Li, and was virtually free of corticotropin, thyrotropin, gonadotropins, and posterior lobe principles, and contained only traces of luteotropin, adipokinin, and intermedin. Unlike any growth hormone preparation previously studied, it was found to evoke anabolic effects in man. Intramuscular injection caused retention of nitrogen and phosphorus in a 26-year-old woman with hypopituitarism. Despite a daily intake of only 7.9 grams of nitrogen and 727 milligrams of phosphorus, the average daily excretion during eleven days of increasing doses of growth hormone was 6.7 grams of nitrogen and 686 milligrams of phosphorus as compared with 7.8 grams and 912 milligrams during ten control days. Thirty milligrams per day appeared to be an effective dose. Neither glycosuria nor increase in fasting blood sugar concentration occurred with 240 milligrams per day for six days.

Studies with this preparation did not confirm the current view that the diabetogenic effect of pituitary extracts is due to growth hormone. Daily injections in adult dogs failed to produce glycosuria. Responsiveness of the animals to pituitary diabetogenic effect was demonstrated by the production of glycosuria with Armour growth hormone in one-quarter the dose. Glycosuria disappeared on changing from Armour material to the larger dose of the present preparation.

Dosimetric Considerations with Radioiodine. J. E. RALL, CHARLES E. FOSTER and WENDELL PEACOCK, New York, New York. (Introduced by Rulon W. Rawson).

The dynamic distribution of 29 large doses of I^{131} (50 to 300 millicuries) given to 24 patients with cancer of the thyroid and the effect of this amount of radiation on the organism have been studied. The only serious effect of this radiation was manifested by the hematopoietic tissues.

A satisfactory dose response curve was obtained by a plot of fall in lymphocytes against blood radiation calculated as the integrated blood concentrated plus gamma radiation from I^{131} localized in the tumor. Due to the marked variation between patients in the rates of turnover of iodine it was not possible to predict the effect of any given dose without prior tracer studies. Suitable

study of a previous tracer dose of radioiodine however permitted fairly accurate estimation of the response to a large dose.

With the above data it has been possible to calculate the relative advantages of the 4-day, 8-day and the 13-day isotopes of iodine in producing a more favorable tumor blood radiation ratio.

Antibody Formation in Surviving Tissues. HELEN M. RANNEY and IRVING M. LONDON,* New York, N. Y.

This study is designed to investigate the participation of various tissues in antibody formation by determining the capacity of surviving tissues to form antibody. The technique which has been developed avoids the difficulty of determining whether antibody present in a tissue has been synthesized by that tissue or whether it has been transported to the tissue from its origin at another site. Rabbits were immunized for several weeks with a vaccine of killed pneumcocci of a single type. Twelve hours before the animals were sacrificed, they were passively immunized with an antiserum to a pneumococcus of a type different from that used for the active immunization. Slices of surviving tissues were prepared, serum containing antibodies to both types of pneumococci was added as inert carrier, and the preparations were incubated with C^{14}-labeled glycine. The formation of antibody *in vitro* could be expected to utilize some of the labeled amino acid so that the newly formed antibody would be labeled isotopically whereas preformed antibody would not be labeled. After incubation, tissues and media were separated by centrifugation, and large amounts of non-isotopic glycine were added to the supernatant solutions which were then dialyzed against 0.85% sodium chloride. The passively administered antibody and the antibody formed on active immunization were isolated successively by addition of type-specific pneumococcus polysaccharides. The radioactivity of the dried specific precipitates was determined.

Significant radioactivity was found in the specific precipitates of actively formed antibody in spleen, bone marrow, lung, and liver. The absence of significant radioactivity in the specific precipitates of passively administered antibody indicates that non-specific adsorption or co-precipitation of isotopically labeled products does not account for the isotope activity observed in the actively formed antibody. Thus far, using only the pneumococcus as antigen, we have not observed antibody synthesis by heart or kidney tissue.

Pituitary Respiratory-Quotient Depressing Factor. LILLIAN RECANT, St. Louis, Missouri. (Introduced by Thomas H. Hunter).

The nature and locus of action of the pituitary respiratory-quotient (R.Q.) depressing factor has been studied. In a Ringer-phosphate medium, the R.Q. of a normal fasted rat diaphragm is .68. In a glucose medium the R.Q. rises to .83. The *in vitro* addition of certain anterior pituitary preparations to the medium depresses the glucose R.Q. to .68. This effect is not reversed by insulin *in vitro*. Of the preparations tested, crystalline growth hormone showed the greatest activity. Though similar in many properties, the R.Q. factor does not appear to be identical with the growth hormone since loss of R.Q. activity may occur without change in growth activity.

On the addition of glucose, hexosediphosphate, 3-phosphoglyceric acid, pyruvate, or acetate, the diaphragm R.Q. increased to values approaching theory. Growth hormone (100 micrograms/ml. medium) inhibited the rise in R.Q. only in the case of glucose and hexosediphosphate. Although no decrease in glucose or hexosediphosphate uptake occurred, lactate production from hexosediphosphate was significantly decreased. These data suggest that the R.Q. factor inhibits the transformation of hexosediphosphate to pyruvate and lactate.

An R.Q. depressing factor has been demonstrated in the serum of fasted normal rats but is absent in fasted hypophysectomized and fed normal rats. Correlation of glycogen and serum factor levels suggests a carbohydrate conserving function for the R.Q. factor, compatible with the above noted site of action. Serum studies will be extended to include normal and diabetic patients.

The implications of these observations with respect to human physiology will be discussed.

The Metabolic Effects of Compound F Acetate in Man. ARNOLD S. RELMAN and WILLIAM B. SCHWARTZ, Boston, Massachusetts. (Introduced by Chester S. Keefer).

Balance studies with 17-hydroxycorticosterone acetate (Compound F acetate) have been carried out in a small group of male patients with normal adrenal function and in two with hypoadrenalism. When given intramuscularly in doses of approximately 200 mgm/day Compound F acetate produced mininal effects on electrolyte metabolism. When given orally in doses of 200–300 mgm/day the steroid produced large positive balances of sodium and chloride for several days after which no further retention occurred. There was initially a marked negative potassium balanced which in a few days rapidly decreased despite continued treatment. There was a large sustained negative nitrogen balance which soon exceeded the daily loss of potassium as predicted from the usual K:N ratio. Urinary excretion of ammonia and phosphorus was increased but there was no significant change in urine titratable acidity. The serum bicarbonate rose slightly and the serum potassium decreased slightly. Glucose tolerance became impaired but there was no glycosuria. There was a small rise in excretion of total organic acids. Following withdrawal of Compound F acetate there was a negative balance of sodium and a positive balance of potassium.

In one short-term preliminary experiment, rigid sodium restriction appeared to modify the effect of Compound F acetate on excretion of postassium, phosphate, nitrogen and ammonia.

Serial determinations of I^{131} uptake by the thyroid and assays of urinary 17-ketosteroid excretion suggested that Compound F acetate depressed pituitary release of thy-

rotrophin and corticotrophin. In hypoadrenal subjects administration of the steroid increased urinary 17-ketosteroid excretion.

The Intravenous ACTH Test. ALBERT E. RENOLD, JOSE GARCIA-REYES and DALTON JENKINS, Boston, Mass. (Introduced by George W. Thorn).

The capacity of the adrenal cortex to respond to its physiological stimulus as determined by ACTH administration constitutes the most specific measure of adrenocortical reserve. Quantitative studies have demonstrated: (a) adrenocortical activation by intravenous infusion of a given dose of ACTH is directly proportional to duration of infusion; (b) stimulation obtained over a given time is directly proportional to ACTH dosage up to a critical amount. Based on the derived log dose-response curve, an intravenous ACTH test has been standardized: ACTH, 20 I. U. in 500 ml. normal saline, or 5 per cent dextrose, infused for 8 hours on two successive days. Indices utilized in evaluating adrenal response are: (a) changes in circulating eosinophils, (b) 24-hour excretion of 17-ketosteroids.

In preliminary studies, one hundred normal subjects and patients without adrenal disease exhibited a mean eosinopenia of 97 per cent (range 80–100) and a mean 17-ketosteroid rise of 5.4 mg. per day (range 0.9 to 19). Only six subjects sustained an eosinopenia less than 90 per cent. The wide range of 17-ketosteroid values necessitates the administration of ACTH on two successive days. Results of this test in 35 cases reveal a mean 17-ketosteroid rise of 4.4 mg. per day on the first day and 8.7 mg. (range 4.7–16) on the second. Thirty Addisonians showed a mean *rise* in eosinophils of 0.6 per cent (range $-29-+40$) and a mean 17-ketosteroid fall of 0.2 mg. (range $-2.9=+1.9$). Twelve patients following bilateral adrenalectomy exhibited a mean eosinopenia of 5 per cent (range $-26-+20$) and a mean 17-ketosteroid rise of 0.2 mg per 24 hours (range $1.5-+2.9$).

The intravenous ACTH test is the method of choice in evaluating adrenocortical function because of: (a) minimal amounts of ACTH required; (b) maximal stimulation produced; and (c) elimination of the vagaries of intramuscular absorption.

The Effect of Mercurial Diuretics on the Alterations in Renal Tubular Function of Salt-Depleted Dogs. LESTER RICE, JULIAN FRIEDEN, MORTON SMITH and BENJAMIN KAPLAN, Chicago, Ill. (Introduced by L. N. Katz).

Salt-depletion provides a convenient method for effecting increased renal tubular sodium reabsorption. Presumably this increased renal tubular capacity for sodium reabsorption results from enhanced *distal* tubular activity. In an attempt to assess the site of action of mercurial diuretics, a study was made of their influence on renal sodium handling in salt-depleted dogs.

During infusion of 1.5% sodium chloride solution, salt-depleted dogs exhibited two consistent differences in response to saturation doses of mercuhydrin (2 cc. intravenously) when compared with these animals in normal sodium balance, viz., (1) the per cent of filtered sodium excreted was lower and (2) the sodium concentration of the reabsorbate was higher than in the controls. Thus mercurialization did not abolish the differences in renal tubular function between dogs in the normal and the salt depleted state. If facultative sodium reabsorption is confined to the distal tubule, these findings suggest that the primary site of action of the mercurial diuretics is the proximal tubule.

Variations in Renal Tubular Reabsorption of Sodium Independent of Change in Adrenocortical Hormone. JACK D. ROSENBAUM, SOLOMON PAPPER and MILTON M. ASHLEY, Boston, Mass. (Introduced by Henry Jackson, Jr.).

Because adrenocortical hormones exert important influences upon the renal tubular reabsorption of sodium, it has often been suggested that physiologic alterations in sodium excretion may be mediated by changes in endogenous adrenocortical activity. However, in patients with Addison's disease, who lack an endogenous source of adrenocortical hormone, the provision of a constant exogenous supply of desoxycorticosterone acetate and cortisone must establish an essentially uniform hormonal level. In such patients, therefore, large variations in tubular reabsorption of sodium could not reasonably be attributed to changes in the amount of available hormone.

In two patients with Addison's disease, maintained on desoxycorticosterone acetate and cortisone, variations in renal excretion of sodium were studied in relation to (a) change in dietary sodium intake, (b) diurnal excretory rhythm, (c) change in posture, (d) venous congestion of the limbs, (e) hypotonic expansion of extracellular fluid volume (by intravenous infusion of a mixture of sodium chloride and sodium bicarbonate). Under these conditions both patients manifested large changes in excretion of sodium indistinguishable from those observed in normal subjects and independent of alterations in filtered sodium load or endogenous creatinine clearance. These observations indicate that appropriate renal tubular responses to stimuli for sodium conservation or excretion do not require a *change* in the level of adrenocortical activity.

Hypotonic expansion of extracellular fluid volume was carried out in one of the patients while he was maintained on desoxycorticosterone acetate alone, prior to the administration of cortisone. His failure to manifest a natriuretic response under these circumstances suggests that, although such a response does not require a *change* in adrenocortical activity, an adequate supply of cortisone-like steroid is required to maintain the integrity of the mechanism involved.

Behavior of Lipids during Electrophoresis of Serum on Paper. I. N. ROSENBERG, Boston, Massachusetts. (Introduced by Samuel Proger).

The association of serum lipids with proteins was studied by performing electrophoresis of human serum on filter paper and comparing the resulting protein and lipid pat-

terns. Serum (0.1 cc.) was applied as a narrow band across a 40 × 11 cm. sheet of Whatman 3 MM paper, which was then saturated with barbital buffer (.025 M, pH 8.6) and a potential of 320 volts (4.4 milliamperes) was maintained between the ends of the paper for four hours. Longitudinal parallel strips of the dried paper were then stained separately. Proteins were demonstrated by brom phenol blue (Durrum). Fat was stained by Sudan IV (Rossman) and cholesterol either by spraying with antimony pentachloride or by the Schultz reagent (1:1 acetic acid-sulfuric acid) applied to paper pretreated with ferric chloride.

The microelectrophoretic protein patterns indicated four bands tentatively identified as albumin, alpha-, beta-, and gamma-globulin. Sudan stains showed that most of the lipid corresponded closely with the beta-globulin band, much smaller amounts being present in the albumin fraction, and virtually none in the gamma- and alpha-globulins. In some instances a light trail of sudanophilic material extended from the origin to the beta-globulin position, where it was more concentrated. The Schultz and Sudan patterns were similar, most of the cholesterol being in the beta-globulin region.

Intravenous administration of heparin to fasting subjects and to those with alimentary hyperlipemia induced a rapid and marked but transient change in lipid pattern. This was characterized by an increased extent of both sudanophilic and Schultz-reacting zones toward the anode and beyond the beta-globulin band as compared with the pre-heparin pattern. No change occurred in the serum protein pattern.

Comparative Metabolic Effects of the Intramuscular Use of Cortisone Acetate and 17-Hydroxycorticosterone. (Compound F) Acetate. ROBERT M. SALASSA, MARSCHELLE H. POWER, HAROLD L. MASON and RANDALL G. SPRAGUE,* Rochester, Minnesota.

A comparison was made of the metabolic activity of cortisone acetate and 17-hydroxycorticosterone acetate, administered intramuscularly for prolonged periods, in 3 patients with rheumatoid arthritis. Two of the patients received 3.6 gm. of cortisone acetate in twenty-four days on one occasion, and 3.6 gm. of 17-hydroxycorticosterone acetate in twenty-four days on another. The third patient received 4.2 gm. of cortisone acetate in thirty days on two different occasions, and 4.2 gm. of 17-hydroxycorticosterone acetate in thirty days on another. Nitrogen balance and urinary excretion of 17-ketosteroids and corticosteroids were determined.

Both steroids caused an increase in urinary nitrogen. The effect of 17-hydroxycorticosterone acetate on nitrogen balance was slower in onset and less intense than that of cortisone acetate. The decrease in urinary nitrogen after discontinuance of steroid therapy was slower in the case of 17-hydroxycorticosterone acetate. The total effect of 17-hydroxycorticosterone acetate on nitrogen balance was less than that of cortisone acetate.

Evidence that the foregoing differences in metabolic activity of the two steroids were related at least in part to different rates of absorption from the sites of injection was obtained from studies of the urinary corticosteroids and 17-ketosteroids. The increase in urinary corticosteroids after administration of 17-hydroxycorticosterone acetate was slower and less pronounced than it was after administration of cortisone acetate. Urinary corticosteroids decreased more rapidly after discontinuance of administration of cortisone acetate than after discontinuance of the administration of 17-hydroxycorticosterone acetate. The urinary 17-ketosteroids increased after administration of cortisone acetate, but this effect was less marked or absent after administration of 17-hydroxycorticosterone acetate.

It is concluded that the observed differences in metabolic effects of the two steroids under the conditions of this study are quantitative rather than qualitative, and probably are related at least in part to slower absorption of 17-hydroxycorticosterone acetate from the sites of injection.

Favorable Effects of L-Norepinephrine on Experimental Localized Ischemia of the Myocardium. JOHN J. SAYEN, WARNER F. SHELDON, PETER T. KUO, HARRY F. ZINSSER, ORVILLE HORWITZ and ALI F. SUMEN, Philadelphia, Penna. (Introduced by Charles C. Wolferth).

In areas of myocardial ischemia produced by coronary obstruction, small doses of l-norepinephrine cause striking rises in oxygen tension. This has been demonstrated by polarographic estimations of intramyocardial oxygen tension made at multiple areas in the exposed left ventricles of anesthetized dogs.

Coronary occlusion or severe narrowing is followed by a progressive fall in local oxygen tension, failure of muscle contraction and later characteristic changes in the epicardial electrocardiogram. Intravenous injection of 0.5 microgram per kilogram of l-norepinephrine reverses these evidences of ischemia, while producing marked bradycardia and a slight increase of mean blood pressure but no ventricular ectopic beats. The myocardium outside the ischemic zone shows unchanged or slightly decreased oxygen tension. With single doses favorable changes are transient, but can be reproduced by repetition of the dose or maintained by a slow infusion during prolonged coronary obstruction. After Regitine (C-7337), which partially abolishes both the bradycardia and the pressor effect of l-norepinephrine, clear-cut beneficial effects in ischemic areas fail to occur.

L-norepinephrine produces greater and more rapid favorable changes in ischemic areas than pure oxygen inhalation. However, oxygen increases the improvement when administered simultaneously. Both agents in combination may produce rises of oxygen tension in large ischemic areas which have failed to show favorable responses to either agent alone.

In contrast to l-norepinephrine, l-epinephrine in small doses causes ectopic beats and does not produce definite improvement in ischemic areas. Both drugs in larger

dosage produce severe hypertension and frequent ectopic beats. While such doses increase the oxygen tension of myocardium which has a normal blood supply, they not only fail to reverse, but sometimes accentuate, the evidences of ischemia in muscle supplied by an obstructed coronary branch.

The Changes in Cerebral Vascular Tone in Experimental Metabolic Alkalosis and Acidosis. JAMES F. SCHIEVE and WILLIAM P. WILSON, Durham, North Carolina. (Introduced by J. M. Ruffin).

The increased cerebral blood flow (CBF) of respiratory acidosis produced by inhalation of carbon dioxide is associated with decreased pH and increased carbon dioxide content of arterial blood. The decreased CBF of respiratory alkalosis following hyperventilation is associated with increased pH and decreased carbon dioxide. It is stated that cerebral vascular tone is predominantly under chemical control, and that, in absence of anoxia, this tone is regulated by either pH or carbon dioxide content of arterial blood. Previous studies fail to separate which of these two factors is of prime importance.

Metabolic alkalosis and acidosis were established by intravenous infusion of $NaHCO_3$ and NH_4Cl, respectively. CBF (nitrous oxide technique) was measured before and during infusions. NH_4Cl caused mild hyperpnea and, if infused too rapidly, nausea. No other symptoms were noted. Infusions of isotonic and hypertonic NaCl served as controls.

Infusions of isotonic NaCl caused no change in CBF or metabolism. Hypertonic saline caused a 10% increase, which was of doubtful statistical significance. Three per cent $NaHCO_3$ produced a 70% increase in CBF without changing cerebral oxygen consumption. This increase is comparable to that found while breathing 5% CO_2, but is without the distressing side effects of hyperpnea or hypertension. When these alkalotic subjects breathed 5% CO_2, there was a further increase in CBF while pH shifted back toward control levels. NH_4Cl caused a 20% decrease in CBF.

Metabolic alkalosis ($NaHCO_3$) increases CBF, and is accompanied by increased pH and CO_2 content, while metabolic acidosis (NH_4Cl) decreases CBF and is accompanied by decreased pH and CO_2 content of arterial blood. The CBF changes are reversed from those reported in respiratory alkalosis and acidosis, and are related to changes in CO_2 rather than pH. This study suggests that CO_2 is more important than pH in regulating cerebral vascular tone.

Experimental Porphyria in Rabbits. RUDI SCHMID and SAMUEL SCHWARTZ, Minneapolis, Minnesota. (Introduced by C. J. Watson).

We have recently observed that in congenital or photosensitive porphyria, the abnormal porphyrin formation originates in the normoblastic nuclei of the bone marrow (porphyria erythropoietica), whereas in the intermittent acute and the "mixed" types the porphyrins originate in the liver (porphyria hepatica). A condition similar in many respects to the human erythropoietic porphyria, with high concentration of uroporphyrin I in the bone marrow, circulating erythrocytes and urine can be achieved by treating rabbits with a combination of phenylhydrazine, intravenous lead acetate and ultraviolet light.

It has now been found that a condition with the essential features of human hepatic porphyria can be produced in rabbits by oral administration of allylisopropylacetylcarbamid (Sedormid). Such rabbits excrete urinary uroporphyrin and porphobilinogen even in excess of the amounts found in the human disease. The uroporphyrin is of the Waldenström type (methyl ester M.P. 252°C), on decarboxylation yielding approximately equal amounts of coproporphyrin I and coproporphyrin III. In further similarity to the human disease the increased urinary coproporphyrin is principally isomer type III and, like the uroporphyrin, it is excreted as the zinc complex. The bile contains enormous concentrations of copro- and protoporphyrin and has intense red fluorescence. The liver exhibits intense red fluorescence and contains high concentrations of uro-, copro- and protoporphyrin as well as porphobilinogen, whereas the bone marrow and the erythrocyte porphyrin concentrations are within the normal limits.

These findings have obvious implications with respect to human porphyria and porphyrin metabolism in general. They demonstrate for the first time that an hepatic as well as an erythropoietic porphyria can be produced experimentally in the rabbit. They further indicate the likelihood that the liver is able to produce porphyrins independently of the porphyrin formation related to hemoglobin synthesis in the bone marrow.

Comparative Studies on the Concentration of β-Alanyl Dipeptides in Skeletal Muscle and Heart. GERHARD SCHMIDT and RICARDO CUBILES, Boston, Massachusetts. (Introduced by Raymond D. Adams).

The β-alanyl peptides, β-alanyl-histine (carnosine) and β-alanyl-1,5-methyl histidine (anserine) are normal constituents of the skeletal muscle of all higher animals. It was of interest to compare the concentration of these β-alanyl peptides in skeletal muscle and heart. The available methods permit quantitative determinations of carnosine but not of anserine. A chromatographic procedure for the determination of the total β-alanyl-peptides and of the free β-alanine was developed in this laboratory. Protein-free tissue extracts were divided into two aliquots, one (A) serving for the determination of free β-alanine, the second (B) for that of bound β-alanine. B was refluxed for 2 hours in 2N-sulfuric acid. After removal of the sulfuric acid with an anion exchanger, the combined filtrate and washings were concentrated under reduced pressure to a small volume. Ascending chromatograms of aliquots of A and B were obtained with a mixture of 75 volumes of butanol and 25 volumes of 50 per cent acetic acid and developed by spraying with a 0.5 per cent solution of ninhydrin in butanol. The blue spots corresponding to β-alanine were extracted with a 50 per cent aqueous solution of acetone, and the

extinction of the acetone solutions was determined colorimetrically.

Although the concentration of bound β-alanine in rat skeletal muscle averaged between 100 and 140 mg per 100 gm of moist tissue, no appreciable amounts of bound β-alanine were found in hearts of rat, rabbit, and beef.

Only very small, if any, amounts of free β-alanine were found in skeletal muscle. No free β-alanine was found in heart tissue.

The Use of the Aromatic Diamidines for Therapy of Systemic Disease Caused by Pathogenic Fungi. EMANUEL B. SCHOENBACH * and JOSEPH M. MILLER, New York City, New York.

Low concentrations of the aromatic diamidines have been shown to inhibit the growth of various species of fungi *in vitro*. Stilbamidine (4,4' stilbenedicarboxamidine), which has been used for the treatment of human trypanosomiasis and leishmaniasis by parenteral administration, has been administered to four patients with systemic blastomycosis, one patient with actinomycosis and two patients with torula meningitis.

No evident response was noted in the torula infections but prompt clearing and sterilization of lesions, associated with symptomatic improvement, were observed in the patients with blastomycosis and actinomycosis. A dosage of 150 mg per day given in three courses to a total of 4.5 to 6 grams appears to be sufficient. In some cases local application of propamidine [p,p' (trimethylenedioxy) dibenzamidine] in 0.1 per cent solution, streptodornase and streptokinase have been employed to supplement the parenteral therapy. The only toxic reaction noted has been the development of a late stilbamidine neuropathy.

Further investigation of the aromatic diamidines for the therapy of systemic involvement with pathogenic fungi is warranted.

Mechanism for Control of Hypertension. HENRY A. SCHROEDER,* H. MITCHELL PERRY, JR and JOHN D. MORROW, St. Louis, Missouri.

Hydrazinophthalazine can act as a carbonyl reagent and probably inactivates pherentasin, the pressor principle found in hypertensive blood, through combination of hydrazine and ketone groups. This drug is relatively non-toxic and produces a moderate lowering of blood pressure most marked in patients who have had unsuccessful sympathectomy. When autonomic ganglia are blocked by hexamethonium halide an additional factor in the production of hypertension appears to be neutralized. While neither drug alone gives extended control of hypertension at reasonable levels, both together are usually effective in this regard. Of more than 60 patients with severe hypertension of all types, followed for 3 to 7 months, continuous combined therapy controlled all at reasonable levels and half at normotensive levels. In all except one of 30 cases of malignant hypertension the malignant stage reversed itself, but only one-third attained normotensive levels. Apparently cholinergic fibers must be blocked by the regimen to be effective since hexamethonium halide cannot be replaced by dibenamine or dihydroergot preparations.

If hydrazinophthalazine or hexamethonium halide is given orally for the control of human hypertension, urinary excretion of the unchanged compound begins quickly, and reaches a maximum in several hours; however, total recovery is no more than 5 or 10 per cent of either. After parenteral administration, half or more of the hexamethonium halide is excreted in the urine, whereas there is no increase in the hydrazinophthalazine recovered. The hydrazine moiety of the latter combines in vitro with sulfhydryl or carbonyl groups of several physiologic substances including cysteine, glutathione, and pyruvic acid. That sulfhydryl binding actually occurs in vivo is demonstrable by breaking this linkage in urine of patients and thereby more than doubling hydrazinophthalazine recovery.

Balance Studies in Two Remarkable Instances of "Pure" Chronic Potassium Depletion Resulting from the Overuse of Laxatives. WILLIAM B. SCHWARTZ and ARNOLD S. RELMAN, Boston, Massachusetts. (Introduced by Joseph H. Pratt).

Prolonged balance studies were conducted on two young women who had gradually developed severe potassium depletion as a result of chronic diarrhea induced by overuse of laxatives.

Despite the loss of approximately one-third of total normal body potassium content (as estimated by correlation of potassium retention with K^{42} dilution data), neither of the patients had overt symptoms or neuromuscular signs. It was only through the discovery of S-T and T wave changes in routine electrocardiograms that hypokalemia was suspected. One patient had a slight increase in serum bicarbonate, and neither had any other disturbance of serum electrolytes except for extreme hypokalemia.

When laxatives were withdrawn, both patients, while on a normal oral intake of potassium and a low normal intake of phosphorus, retained 12 mEq/kgm. of potassium in excess of nitrogen over a period of two weeks, and showed no significant change in the corrected balance of phosphorus. The increase in total exchangeable potassium measured by K^{42} closely predicted the actual potassium retention.

In neither case was there any appreciable change in body weight, nitrogen balance, sodium balance, or extracellular tonicity at the end of the period of potassium retention, suggesting that potassium repletion was associated with a reduction in the osmotic activity of some intracellular constituent. Calculations based on the chloride space indicated a large increase in the intracellular concentration of potassium and total cation and a shift of sodium from cells to the chloride space of a magnitude nearly equal to the cellular uptake of potassium.

After potassium retention was complete, when serum potassium and the electrocardiogram had returned to normal, readministration of laxative to one subject produced diarrhea and a negative potassium balance. The electrocardiogram again became abnormal. This change occurred prior to the fall in serum potassium.

Blood Ketoglutarate and Pyruvate in Liver Disease. DAVID SELIGSON, GERALD J. MCCORMICK and VICTOR SBOROV, Washington, D. C. (Introduced by Francis C. Wood).

The specific measurement of alpha-ketoglutaric acid (KG) and pyruvic acid (P) in blood is made possible by a new method using paper chromatography to separate their dinitrophenylhydrazones from other ketones normally present. Because of the known importances of KG and P in intermediary metabolism these substances were measured in liver and other diseases to establish a possible relationship between these measurements and hepatic disease.

Eighteen normal males showed a mean KG blood level of 13.0 ± 1.5 micromoles/liter. The mean level of P in these individuals was 73 ± 16 micromoles/liter. Values above two standard deviations were considered abnormal ($KG > 16.0$ and $P > 105$). Sixteen of eighteen cases of acute viral hepatitis showed abnormal KG values whereas only five showed elevated P values. The means were 24.0 ± 11.7 and 91 ± 26 respectively. Of sixteen patients with chronic viral hepatitis thirteen had abnormal KG and five had abnormal P levels. Of nineteen cases with cirrhosis sixteen had abnormal KG and eleven abnormal P levels.

A series of miscellaneous diseases involving the liver showed abnormally high KG which paralleled other evidences of hepatic dysfunction. Jaundiced patients without liver damage showed normal values.

Serial determinations of KG and P were made in acute viral hepatitis. Although the peak elevation of KG occurred later than the peak of bilirubin, KG tended to fall with recovery. Fewer of the PA values were abnormal in this group; their fall, however, paralleled the KG values with recovery. Wide variations of KG and P occurred in the cirrhotics usually associated with therapy, physical activity or change in severity of the disease.

It is concluded that liver damage is associated with an elevation of blood KG and to a lesser extent pyruvate. It is believed that in liver disease this reflects a defect in intermediary metabolism.

Barbiturate Tolerance Tests in Patients with Liver Disease. J. T. SESSIONS, JR. and F. J. INGELFINGER,* Boston, Massachusetts.

Clinical studies and liver function tests made by us in patients with stabilized cirrhosis have not substantiated the belief that these patients are highly susceptible to hypnotic doses of pentobarbital. To obtain information on the handling of barbiturates by cirrhotic patients, an attempt was made to measure pentobarbital blood levels by a spectrophotometric technique (239–240 mμ), but this proved unsuccessful following single parenteral doses of the drug. During and following constant infusions of 100–120 mg. of pentobarbital, however, rising and falling blood levels were measured satisfactorily. The tolerance curves so obtained in 10 control subjects and in 13 patients with advanced cirrhosis were similar. It seems that the disappearance from the blood of pentobarbital, or early breakdown products, is not affected by hepatic disease.

Barbiturates may diffuse rapidly out of the blood, especially since they are said not to be bound to serum proteins. To test this possibility, simultaneous measurements of pentobarbital were made on blood and ascitic fluid during a constant barbiturate infusion. The tolerance curves obtained in the two compartments were nearly parallel. The increased fluid volume in patients with ascites and the ready diffusibility of pentobarbital may influence barbiturate tolerance curves in such patients.

Pyruvate metabolism is said to be affected both by barbiturates and by liver disease. We have confirmed the finding that blood pyruvate levels tend to be increased in patients with hepatic disease, but constant infusions of pentobarbital did not change these levels.

Studies on the Biologic Significance of the Protection Afforded Intracellular Brucella. JAMES M. SHAFFER and CARRELL J. KUCERA, Minneapolis, Minn. (Introduced by Wesley W. Spink).

Localization of Brucella intracellularly in infected animals or man constitutes an excellent defense mechanism for the bacteria against therapeutic agents or immune mechanisms. An experimental technique to study this problem utilized exudative leukocytes containing large numbers of intracellular Brucella obtained by the *in vivo* phagocytosis of Brucella in the peritoneal cavity of adult male white rats. These preparations maintained *in vitro* in a synthetic tissue culture medium revealed a remarkable protection of intracellular Brucella against therapeutic agents, when used alone or in combination. Viable streptomycin-sensitive Brucella have been recovered from leukocytes exposed for 24 hours to 25,000 times the minimal concentration of streptomycin required to kill the organism in a broth culture. None of the therapeutic agents now available for clinical use, whether alone or in combinations, were found to be capable of killing all the intracellular Brucella in 24 hours. Streptomycin combined with aureomycin, terramycin, chloramphenicol, or sulfadiazine was more effective than any single therapeutic agent. This protection of intracellular Brucella has been found to be dependent upon intact, viable leukocytes.

Reduction of Neotetrazolium by leukocytes and Brucella has been investigated as a means of evaluating the metabolic status of the cells and parasite before and after exposure to therapeutic agents. This method can be used to differentiate viable from nonviable leukocytes and heat-killed or phenol-killed Brucella from organisms exposed to high concentrations of the antibiotics.

Phagocytosis of Brucella favors the host by clearing the blood stream of organisms and localizing the infection. Intracellular localization favors the bacteria by maintaining them in a viable state and affording protection against attempts to eradicate the organisms from the tissues. This biologic phenomenon accounts in part for the the chronicity of the disease and relapses that occur.

The Separation of Specific Serum Proteolytic Inhibitors and the Physiological Significance of Increased Proteolytic Inhibition. N. RAPHAEL SHULMAN, Bethesda, Maryland. (Introduced by Thomas McP. Brown).

Increased serum antiproteolytic activity occurs in association with various diseases. It has generally been assumed that inhibition of trypsin, chymotrypsin, and plasma (fibrinolysin) is caused by a single substance in serum which occasionally increases in disease. The significance of the increase is not understood for there have been no previous reports of changes in accurately measurable biological phenomena occurring consistently with changes in proteolytic inhibition.

A method of measuring proteolytic activity using a radio-actively tagged substrate was developed to permit highly sensitive measurements in media such as serum. At least two separate anti-proteolytic factors were detected in normal serum by differences in their physical and chemical properties and the specificity of their action on the enzymes tryspin, chymotrypsin, and plasmin. One factor specifically inhibited plasmin (plasmin inhibitor) while the other factor inhibited both trypsin and chymotrypsin (trypsin inhibitor). The inhibitors could be separated by chemical fractionation of serum. Increases of proteolytic inhibition with disease were specific either for plasmin or for trypsin and chymotrypsin.

An analysis was made of the relationship of the level of proteolytic inhibition to the clinical and laboratory data on 117 patients with cancer, various acute diseases, and tuberculosis. It was found that elevated trypsin inhibitor is associated with disease processes that bring about cellular destruction and that there is a close correlation between changes in trypsin inhibitor and changes in sedimentation rate. Intravenous typhoid vaccine was found to produce parallel changes in trypsin inhibitor and fibrinogen concentration in humans. It was concluded that the diagnostic significance of increased trypsin inhibition (and chymotrypsin inhibition) is the same as that of increased fibrinogen concentration (or sedimentation rate).

The question of why trypsin inhibitor and fibrinogen concentration vary together and the circumstances which lead to increases in them will be discussed.

The Effect of Negative Pressure Breathing on Renal Function. H. O. SIEKER, O. H. GAUER and J. P. HENRY, Dayton, Ohio. (Introduced by R. W. Rundles).

Previous animal studies have suggested that receptor areas in the low pressure side of the cardiovascular system within the thorax appreciate changes in effective blood volume and alter urine flow (Henry and Gauer, 1951; Gauer et al, 1951). Decreasing the blood volume in the thoracic viscera by positive pressure breathing is associated with oliguria in humans (Drury et al, 1947). This study was undertaken to determine in man the effect of negative pressure breathing on renal function.

Following five hour control periods, recumbent normal subjects pressure breathed at -15 cm. water for 45 minutes. Urine flow, pH, sodium excretion, and glomerular filtration rate were measured. Blood pressure, pulse rate, and respiration rate were also followed. During negative pressure breathing the urine flow showed an average increase of 340 per cent. The urine pH during this period was unaltered or inconsistently changed. The urine sodium concentration during negative pressure breathing decreased an average of 73 per cent and the total sodium excreted increased only 17 per cent. In 3 of 7 subjects the total sodium excreted during this period did not increase or it fell below control levels. The glomerular filtration rate increased an average of 42 per cent initially but returned to the control value as negative pressure breathing was continued. The pulse rate, blood pressure, and respiration rate were not significantly altered.

The marked diuresis cannot be attributed to alkalosis because the respiration rate and urine pH were unaltered or inconsistently changed and there was only a slight increase in the total urine sodium excretion. It is suggested that the increased urine flow which resembles "water diuresis" is the result of engorgement of receptor areas in the thorax. These receptors, it is thought, mediate changes in renal function through the endocrine glands and renal hemodynamics.

The Effect upon the Liver of Inhalation of Carbon Dioxide in High Concentrations. J. L. SIMS, O. S. ORTH and LUCIEN MORRIS, Madison, Wisconsin. (Introduced by Ovid O. Meyer).

The finding that increased concentration of carbon dioxide in gas mixtures used for surgical anesthesia was associated with increased hepatic functional impairment suggested a study of the effect upon the liver of this agent alone.

A mixture of one third carbon dioxide and two thirds oxygen was administered by intratracheal tube for periods of 25 to 30 minutes to each of ten dogs. Anesthesia and analgesia resulted. Blood pH fell to 6.7-6.8. Needle biopsy of the liver, and tests of bromsulfalein clearance, prothrombin time, icterus index, and prothrombin consumption were performed immediately before and after the administration, and at 24 and 48 hours afterward. The prothrombin consumption showed an irregular variation without definite trend. The results of the other tests and the histologic appearance of the liver remained unaltered.

Five deteriorated schizophrenics in whom other forms of shock therapy had been unavailing were anesthetized for 25 to 30 minutes with a mixture of 25% to 35% carbon dioxide and 75% to 65% oxygen. Cephalin cholesterol flocculation, thymol turbidity, bromsulfalein clearance and icterus index studies performed before, immediately after, and at 24, 48, 72, and 96 hours after the administration showed no change. In two patients prothrombin time increased moderately, and in one slightly. In two it remained unchanged. Bromsulfalein clearance studied during the actual period of anesthetization in two patients was greatly slowed.

It is concluded that carbon dioxide alone, administered in the manner described, produces no significant hepatic damage.

The Effects of Acute Respiratory Alkalosis on Electrolyte Excretion and Renal Hemodynamics in Man. R. B. SINGER, J. K. CLARK,* E. S. BARKER and J. R. ELKINTON,* Philadelphia, Pa.

Voluntary hyperventilation of ½ or 1 hour by 5 normal subjects caused abrupt changes typical of respiratory alkalosis in arterial blood, including average rise in pH of + 0.25, fall in plasma bicarbonate concentration, and fall in calculated pCO_2 to less than 20 mm. Hg.

Average urinary responses in 8–10 collection periods per subject were: 1) Variable urine flow, the most consistent change being > 60% drop during the first period after hyperventilation. This decrease was abolished in one subject by maintaining an osmotic diuresis (mannitol). 2) A characteristic acid-base response with rise in pH to 7.30 and bicarbonate excretion rate to 192 μEq./min., and fall in ammonia output. 3) Rise in potassium excretion rate of + 170 μEq./min., sustained during hyperventilation, and steady fall afterward reaching in the final period 50 μEq./min. below the control. 4) Rise in sodium excretion rate of + 170, and in chloride + 110 μEq./min. in ½ hour of hyperventilation, with subsequent abrupt fall below the control levels, regardless of whether the hyperventilation was continued or not. 5) Decrease in glomerular filtration rate (endogenous creatinine) averaging 13% during and after hyperventilation, with variable changes in effective renal plasma flow (PAH).

Thus, only one-quarter of the urinary acid-base response was due to increase in sodium bircarbonate and decrease in ammonia and titratable acid. Increased potassium bicarbonate excretion played the major role, a finding in agreement with the hypothesis of a relationship between the tubular secretion of hydrogen and potassium. These changes are consistent and correlate well with those in pH of blood and urine. In contrast, the changes in chloride + equivalent sodium excretion appear to be associated with some factor of early hyperventilation which is not blood pH, urine volume, glomerular filtration, or diurnal variability of electrolyte excretion.

Renal Mechanisms of Uricosuria Produced By Benemid (p-(di-n-propylsulfamyl)-benzoic acid) and Salicylate. JONAS H. SIROTA and T. F. YU, New York, N. Y. (Introduced by Alexander B. Gutman).

The effects of Benemid, a potent uricosuric agent for use in chronic gout, upon the renal clearances of urate, inulin, p-aminohippurate and electrolytes were studied and its mechanism of action investigated in 19 gouty subjects. A single 2 gm dose given orally (19.3 to 29.5 mgm/kilo) uniformly produced a marked increase in C_{urate} without affecting C_{inulin}. The C_{urate}/C_{inulin} ratios rose from a mean of 0.0718 (range 0.0502 to 0.103) to peak values averaging 0.337 (range 0.118 to 0.547). The elevations were evident within 40 minutes, attained peak values within 4 hours and persisted for 24 hours. C_{PAH} was moderately depressed by Benemid; $C_{potassium}$ and $C_{phosphate}$ were uninfluenced and C_{sodium} and $C_{chloride}$ were inconsistently increased, particularly in several subjects with very marked uricosuria. Benemid strikingly and apparently quite selectively inhibits tubular reabsorption of urate.

Intravenous sodium salicylate produced little change in C_{inulin}. When plasma salicylate concentrations exceeded 15 mgm %, C_{urate}/C_{inulin} rose markedly; between 5 and 15 mgm % little change in this ratio was noted; below 5 mgm % significant depression of C_{urate}/C_{inulin} was consistently observed. Salicylate infused during Benemid uricosuria produced depression of C_{urate}/C_{inulin} instead of further augumentation, maximum depressions occurring at plasma salicylate concentrations under 15 mgm %. Salicylate interferences with the Benemid uricosuric effect may be competitive.

A patient with marked Fanconi type glomerulo-tubular imbalance was found to have strikingly decreased reabsorption of urate, among other evidences of tubular damage. C_{urate}/C_{inulin} approximated unity (0.984) but did not exceed it; i.e., there was no indication either of tubular absorption or tubular secretion of urate. As anticipated under these circumstances, Benemid had no effect on urate clearance or urinary urate excretion.

A Comparison of the Metabolism of Fructose and Glucose in Hepatic Disease and Diabetes Mellitus. LLOYD H. SMITH, JR., RICHARD H. ETTINGER and DAVID SELIGSON, Washington, D. C. (Introduced by Richard B. Capps).

In order to quantitate rates of fructose utilization in diseases manifesting glucose intolerance, standard intravenous fructose and glucose tolerance tests were performed on normals and patients with hepatitis, cirrhosis, and diabetes. Glucose tolerance was abnormal in 27 per cent of the patients with hepatitis, 50 per cent of the cirrhotics, and all of the diabetics. The fructose tolerance curves appeared grossly similar in all groups. It was found, however, that the disappearance rate of infused fructose had the characteristics of a first order reaction; the rate of disappearance, therefore, could be expressed as time of "half-life." Comparison of disappearance rates so expressed revealed significant group differences. The normal group had a mean "half-life" value of 18.6 minutes, the hepatitis group 20.8 minutes, the cirrhotics 21.8 minutes and the diabetics 23.6 minutes. No correlation in individual patients was noted between rate of fructose disappearance and degree of glucose tolerance.

Serum inorganic phosphorus fell rapidly in each group when fructose was infused and returned toward normal within one hour. Following glucose infusion a more gradual and persistent phosphorus fall occurred. A significant difference in phosphorus fall following glucose was noted in comparing the hepatic patients manifesting glucose intolerance and the mildest diabetics.

Blood levels of lactic, pyruvic and alpha-keto glutaric acids showed only slight increases following glucose, the maximum elevations occurring at 60 minutes. When fructose was given there were more rapid and marked elevations (up to 300%) in all groups.

It is concluded that although the infused fructose disappeared from the blood rapidly, the rate was slightly decreased in patients with hepatocellular disease and diabetes. The lack of correlation with abnormal glucose tolerance, the differences in response of serum inorganic phosphorus, and the characteristic changes of the carbohydrate intermediaries suggest qualitative differences in the metabolic pathways of fructose and glucose.

The Lipid Composition of Ultracentrifugates of Serum from Patients with Acute Hepatitis. J. R. SNAVELY, W. H. GOLDWATER, M. L. RANDOLPH and W. G. UNGLAUB, New Orleans, Louisiana. (Introduced by R. H. Turner).

Employing a quantity ultracentrifugal technic previously described in detail, the distribution of serum lipids of eight patients with acute hepatitis was determined; three of the patients were restudied in early convalescence.

Fasting serum samples were centrifuged at 48,000 r.p.m. for four hours, the column of centrifugate sampled in the liquid state at horizontal levels, and analyses effected for free and total cholesterol, phospholipids, and total fatty acids.

Characteristic changes from the normal pattern were encountered: Deep in the column where phospholipids and cholesterol esters occurred without free cholesterol; both of these lipids were decreased in concentration. Higher, in the medium density zone, where certain low density aggregates composed of cholesterol, phospholipid, and neutral fat accumulate, there was a marked increase in the concentration of all lipids, and this increase was characterized by a relative decrease in cholesterol ester concentration, and a relative increase in neutral fat as compared to that of free cholesterol. In the three patients whose studies were repeated in convalescence, reversion toward the normal pattern was observed.

As we have indicated previously, acute hepatitis is associated with the appearance of increased concentrations of low density lipoproteins. We suggest that this increase in light aggregates rich in neutral fat and poor in cholesterol ester is a manifestation of increased mobilization of depot fat with sluggish removal by the diseased liver, and that the decrease in phospholipids and cholesterol esters not associated with free cholesterol is a manifestation of defective hepatic synthesis.

Response to Psychological Stress in Persons who are Potentially Hypertensive. MAURICE SOKOLOW,* R. E. HARRIS, L. G. CARPENTER, M. FREEDMAN and S. P. HUNT, San Francisco, Calif.

Investigations of patients with essential hypertension have led us to recognize certain personality and behavior patterns characteristic of the disease. Their relevance for etiology, however, remains obscure. A demonstration that abnormal personality reactions, particularly in situations involving stress, exist before the onset of fixed hypertension would add plausibility to the argument that personality factors play a role in the causal chain of events leading to hypertension. To this end, 39 college women who had shown elevations of blood pressure on routine initial physical examinations were matched with 39 controls wtih low normal pressures. It has been shown that more of the former group will develop hypertension in later life, and hence may be assumed to be "pre-hypertensive."

Controlled experimental stress was induced by staging inter-personal situations in which the subjects played assigned roles. One psychodrama involved simple frustration; the second, criticism, rejection, and hostility from an authoritative person. Psychological tests were obtained before and after the psychodramas. Behavior during the experimental situations was observed and recorded. A psychiatrist interviewed the students, attempted to identify their characteristic responses to stress and to infer which were pre-hypertensive. The test-interpreters, observers, and psychiatrist did not know whether the subjects were pre-hypertensive or control until the data had been analyzed.

Most of the procedures showed clearly significant differences between the groups. The pre-hypertensives were typically more hostile and less well controlled than the normals and, in general, less well-equipped to cope with experimental stress.

Although the etiology of essential hypertension is complex, probably involving constitutional and other factors, our data show that the individuals who reacted less adequately to experimentally induced stress were those who demonstrated an elevated blood pressure on the initial college examination and, therefore, may be presumed to react similarly to other situations of stress and ultimately to develop fixed hypertension.

Studies of Mineral Metabolism in Disturbance of Intestinal Absorption. HERTA SPENCER and ISAAC LEWIN, New York City. (Introduced by B. S. Oppenheimer).

The mechanism of apparent faulty intestinal absorption and its effect upon mineral metabolism and electrolyte balance was studied under metabolic conditions in three cases. One patient had a resection of the major part of the small intestine for sarcoma; two patients had ideopathic steatorrhea associated with hypocalcemia and tetany. On a low calcium diet the derangement of mineral metabolism consisted of an abnormally high fecal and an abnormally low urinary calcium excretion, leading to a markedly negative calcium balance. However, this balance became markedly positive by simply increasing the calcium intake. This suggests that intestinal calcium absorption and utilization are not at fault. The great need for calcium was demonstrated also, by the intravenous calcium tolerance test which showed an extremely low 24 hour calcium excretion indicative of the skeletons avidity for this mineral.

Balance studies of sodium and potassium have shown that considerable quantities of electrolytes were lost through the intestinal canal and that these losses could be well correlated with the high fluid content of the

feces. The total electrolyte concentration of the fecal fluids approximated that of a "plasma-ultra-filtrate." This indicates that the primary defect is the loss of fluids with concomitant loss of electrolytes rather than defective intestinal absorption.

Metabolic studies have also shown that the body is able to compensate partially for losses of electrolytes through the intestinal canal by decreased renal excretion of these substances. Chronic metabolic studies and the application of the intravenous calcium test were helpful in elucidating the mechanism of intestinal disturbances. The effect of ACTH on the clinical course and on metabolic changes will be discussed.

Immunologic Aspects of Idiopathic Thrombocytopenic Purpura (ITP). MARIO STEFANINI, JYOTI B. CHATTERJEA and EDWARD ADELSON, Boston, Massachusetts. (Introduced by William Dameshek).

Recent evidence indicates that the thrombocytopenia of ITP may be immunologic in origin. Antiplatelet substances were accordingly searched for in the sera of twenty patients with ITP. Sera were tested against platelets for agglutination, precipitation, lysis, complement fixation, inhibition of physiologic properties of platelets. Various precautions in handling sera and platelets were taken to prevent non-specific platelet agglutination.

Low titer (1:8) platelet agglutinins were found in two patients. A third patient exhibited a high titer agglutinin (1:1280) causing agglutination of human but not animal platelets. The agglutinin had continued activity after submitting the serum to: (a) acidification (pH 5) or alkalinization (pH 9); (b) temperatures ranging from 4° to 66° C.; (c) absorption with gels; (d) filtration through Seitz and dialysis; (e) absorption with human erythrocytes and leucocytes, beef erythrocytes, guinea pig kidney; (f) storage at 4° C. The agglutinin was present in the gamma globulin fraction of serum.

When injected in humans the patient's plasma induced: (a) severe, sustained thromobocytopenia, with circulating large and bizarre platelets; (b) increased number and size of megakaryocytes with vacuolization and lack of platelet production; (c) demonstrable platelet agglutinin in recipient's serum for 12–14 days.

Seven of eleven patients showed significant Forssman-like antibody against sheep erythrocytes. In one, heteroplatelet cold agglutinin was detected.

In all patients with ITP disappearance of injected platelets was prompt. Plasma of several ITP patients produced severe or moderate thrombocytopenic response in normals. Inability to demonstrate antiplatelet agents *in vitro* more frequently emphasizes: (a) need of finer technics for detection of antiplatelet substances; (b) apparently great variability of pathogenetic mechanisms in ITP. Combined studies of this type already indicate the possibility of discriminaing between different types of ITP and of predicting the therapeutic effects of splenectomy.

Observations on the Storage Lesion of the Erythrocyte. ALEXANDER STEVENS, BEVERLY GABRIO, RICHARD CZAJKOWSKI and CLEMENT FINCH,* Seattle, Washington.

Sequential physical and biochemical changes develop in the human erythrocyte when stored *in vitro*. These changes are retarded by a low storage temperature and by the addition of glucose.

The "storage lesion" is not related to cell senescence as it occurs *in vivo*. Young cells are even more vulnerable to storage damage than are old cells. The concentration of leucocytes and the degree of oxygenation have significant effects on the rate of development of the "storage lesion." However, in all instances metabolic alterations attributable to storage appear.

Manipulations with plasma constituents *in vitro* have not appreciably affected the progress of storage changes nor influenced post-transfusion survival. The biochemical characteristics of the "storage lesion" may be rapidly reversed upon reintroduction of the stored erythrocyte into the blood stream. Red cells have been stored for three weeks, transfused, and withdrawn for a second three weeks of storage. The post-transfusion survival after the second period of storage was excellent.

These observations indicate that in the storage of the erythrocyte aging as it occurs *in vivo* is not a limiting factor. While plasma appears inadequate to support normal red cell metabolism, the lesion produced by storage is rapidly reversible *in vivo*.

Effect of Steroid on Potassium Exchange in Blood. HIRSH SULKOWITCH, SUSAN BUTLER and BENEDICT F. MASSELL, Boston, Mass. (Introduced by Louis K. Diamond).

The mechanism by which steroids influence the transfer of potassium between tissue cells, and their surrounding extracellular fluid is not known. Whole blood was employed as a relatively simple in vitro system for studying this problem.

Cortisone was added to freshly drawn heparinized whole blood and incubated with controls in a roller tube device. During the first hour of incubation, there is a transfer of potassium from cells into plasma. The quantity of potassium transferred in the cortisone-containing blood was many times that exchanged in the controls. After the first hour, in both the cortisone containing blood and the controls, there is a transfer of potassium back into the red cell which is maintained to approximately the fourth hour. The general patterns of the cortisone and control curves are similar, but at all times after the zero hour the concentration of potassium in the plasma is much higher in the cortisone containing samples than the controls.

Of the other steroids studies, only testosterone influenced the transfer of potassium.

Physiological Behavior of Actomyosin Strips of Heart Muscle. MAX TAESCHLER and R. J. BING,* Birmingham, Ala.

Strips of heart muscle of 2 mm. diameter and 3-4 cm. in length were dissected from the ventricular muscle of dogs. The muscle was washed for 8 days in 50% glycerol according to Szent-Gyorgyi. From these preparations, muscle fibers of from .4 to .7 mm. diameter and a length of 2 cm. were dissected. The muscle was suspended in an isotonic solution with a cation composition identical to that of heart muscle. Isotonic and isometric contractions were recorded following the addition of .8% of the Na salt of Adenosinetriphosphate (A.T.P.). The work was calculated as the product of contraction times load. Heart muscle preparations behaved differently from skeletal muscle (rabbit psoas), since in the former the tension developed and the speed of contraction were less. When the preparation was loaded and its contraction recorded isotonically, the degree of contraction and work performed were proportional to the logarithm of time. Increasing the initial length by augmenting the load resulted in a greater output of work. However, the speed and amplitude of contraction were decreased. After a certain load (about 120 gms./cm.^{2} fiber area) was exceeded, the work declined. When the muscle strips were stretched to 20% of their original length during extraction, the work output was significantly increased. However, the relationship of load to work remained qualitatively unchanged. Excessive stretching during extraction reduced the work to values observed on the unstretched muscle. The results indicate that many physiological features of the intact heart are inherent properties of the contractile proteins of heart muscle.

The Hemorrhagic Syndrome of Metastatic Prostatic Cancer and its Treatment. HENRY J. TAGNON,* PHYLLIS SCHULMAN, WILLET F. WHITMORE and SHELDON C. KRAVITZ, New York, N. Y.

Bleeding manifestations are a frequent complication of metastatic cancer of the prostate. In many instances, this hemorrhagic tendency is not associated with thrombocytopenia. We have observed that the hemorrhagic syndrome of metastatic prostatic cancer is caused by the presence of a proteolytic enzyme in the blood of the patients. This enzyme proteolyzes fibrinogen and fibrin as well as one or several of the factors entering into the first phase of the coagulation reaction, thereby prolonging the one-stage prothrombin time. The proteolysis of fibrin appears to be the main cause of the deficient hemostasis. The enzyme is present in normal as well as in the cancerous prostatic tissue and we were able to extract also from the metastases. This fibrinolysis constitutes a new example of the release into the blood of an enzyme in metastatic carcinoma of the prostate. The analogy with acid phosphatase is obvious.

This new pathogenesis of the hemorrhagic syndrome of metastatic prostatic cancer led us to the development of a method of treatment based on the use of a specific inhibitor. This could be injected intravenously, was nontoxic, and completely abolished the action of the enzyme *in vivo* as well as *in vitro,* thereby providing an effective treatment of the hemorrhagic tendency. This treatment proved also valuable by making possible surgical treatment in cases with hemorrhagic tendencies.

It is proposed to call this newly recognized syndrome "prostatic fibrinolysis."

Inhibition of Influenza Virus Multiplication by Benzimidazole Derivatives. IGOR TAMM, KARL FOLKERS and FRANK L. HORSFALL, JR.,* New York, N. Y.

Because certain analogs of vitamins are known to affect the metabolic processes of living cells in definite ways, it seemed possible that some might also cause inhibition of influenza virus multiplication. Clear evidence of such inhibition has been obtained with a number of synthetic vitamin analogs. The most interesting substance yet employed is 2,5-dimethylbenzimidazole, an analog of 5,6-dimethylbenzimidazole which is a moiety of vitamin B_{12}. In a concentration of 0.002 M, this compound restricted the multiplication of influenza A (PR8) or B (Lee) virus to about 10 per cent of the control value and did not affect the rate of oxygen consumption of the host tissue.

Experiments were carried out under strictly controlled conditions *in vitro:* Portions of normal chorioallantoic membrane in chemically defined media were incubated in Warburg vessels or in test tubes which were shaken mechanically. After inoculation, maximal viral concentrations, as measured by hemagglutination, were attained in 30 to 40 hours. 2,5-Dimethylbenzimidazole had no inactivating effect upon the infectivity or the hemagglutinating capacity of influenza viruses. Inhibition of multiplication was obtained when the substance was introduced before or after the virus. Attempts to block the inhibition with vitamin B_{12} or riboflavin have failed.

The available evidence suggests that 2,5-dimethylbenzimidazole interrupts the multiplication of influenza viruses through host cell metabolic mechanisms which are not directly related to respiration. The structure of the compound raises the possibility that it may affect nucleic acid metabolism.

Chloride "Space" and "Mass" Measured with Long-Life Radiochloride, Cl^{36}, in Intact Man. S. A. THREEFOOT, G. E. BURCH * and C. T. RAY, New Orleans, Louisiana.

Long-life radiochloride, Cl^{36}, has made possible the determination of chloride space after equilibrium of distribution has been reached (24–120 hrs.).

Chloride space and *chloride mass* were determined daily for 30–60 consecutive days by the isotope dilution method and from curves of biologic decay rates in intact man (two control subjects, two with moderate congestive heart failure and two with severe failure).

Chloride space in serum equivalents was larger in those subjects with failure, especially with edema. The overall average space was 22 and 32% of body weight for the controls, 40 and 50% for those with moderate, and about 60% for those with severe failure. Calculated chloride

mass for the control subjects was 40.5 and 58.9 Gm. (0.79 and 1.24 Gm./kg. body wt.), the patients with moderately severe congestive failure 71.6 and 85.2 Gm. (1.33 and 1.83 Gm./kg. body wt.) and the patients with severe failure 88.7 and 147 Gm. (2.02 and 2.21 Gm./kg. body wt.). Wide daily variations in both the calculated chloride space and calculated chloride mass were especially noticeable in the subjects with failure.

In explanation of the fluctuating nature of calculated chloride space and mass, especially without concordant variations in body weight, it is postulated that the chloride compartments of the body not only are anatomically and functually subdivided but also present time-to-time variations in rate of chloride exchange with the serum compartment. Apparently there is "blinking" in activity of the subcompartments of exchangeable chloride. This "blinking" is influenced by several factors, for example, variations in the state of congestive heart failure, spontaneous and therapeutically induced, and dietary intake of chloride. Data obtained conform with the postulates. These phenomena are complex and readily lead to error in calculation of chloride "space" and "mass."

Changes in the Protein-Bound Iodine and the Clinical Course of Thyrotoxic Crisis Treated with ACTH. BEVERLY T. TOWERY, Nashville, Tennessee. (Introduced by J. Allen Kennedy).

Certain observations suggest that the initiation of thyrotoxic crisis or its severity may be contingent upon an inadequate adrenocortical response to the stress imposed by severe thyrotoxicosis. Further evidence in support of this hypothesis was obtained during the course of an unequivocal thyroid crisis treated with ACTH alone.

The patient was a 32 year old male with uncomplicated thyrotoxicosis. The events which antedated the crisis were: Graves' disease for 10 months; temporary remission under iodide therapy; no therapy for 6 months; recrudescence of thyrotoxicosis; resumption of Lugol's therapy two weeks prior to hospitalization. Upon admission this medication was discontinued and radioiodine therapy was given on the 14th hospital day.

The first evidence of impending crisis appeared on the 17th hospital day with the onset of nausea; shortly thereafter the temperature rose to 101.6° C. and manifestations of thyrotoxic crisis were alarmingly evident.

The intravenous administration of ACTH (10 mg. per liter of dextrose solution) was started immediately and repeated on two successive days. Marked clinical improvement occurred within 18 hours and all manifestations of crisis virtually disappeared within 48 hours.

Coincident with crisis there was a striking increase in the serum protein-bound iodine concentration and a sharp decrease during the exhibition of ACTH. The circulating eosinophils were relatively high during the crisis and completely disappeared in response to the initial dose of ACTH.

The most plausible interpretation of these observations is provided by the conclusion that the thyroid crisis was associated with a lack of sufficient endogenous corticotropin to evoke an adrenocortical response commensurate with the exigency but that such a response could be achieved with exogenous ACTH. The implications of this hypothesis will be discussed in relation to the rise in PBI in thyroid crisis.

The Site of Synthesis of Creatine in Human Subjects. FRANK H. TYLER,* AVERY A. SANDBERG and HANS H. HECHT,* Salt Lake City, Utah.

It has been demonstrated that kidney tissue is capable of making glycocyamine (GAA) from arginine and glycine and that creatine is normally formed by methylation of GAA in the liver. These observations are based on experiments with mammalian tissue slices and homogenates. Because of our interest in the creatine anomaly of progressive muscular dystrophy we desired to determine if this mechanism was true in the human.

Blood samples were obtained from the hepatic and renal veins in six healthy young adult males by vascular catheterization. Simultaneously, arterial samples were obtained, and serum levels of creatine, creatinine, GAA, and arginine were determined in each sample. The averaged values (expressed as mgm. per 100 ml. of serum) obtained in the arterial blood were: GAA 0.23, creatine 0.35, creatinine 0.86, arginine 1.20. Hepatic venous levels were: GAA 0.19, creatine 0.84, creatinine 0.94, arginine 1.08. The values in the renal venous system were: GAA 0.28, creatine 0.43, creatinine 0.73, arginine 1.34.

These data relate only to changes in concentration but they demonstrate that GAA formation by the kidney is small when compared to the drop in GAA concentration which occurs across the splanchnic circulation. The slight increase in GAA and creatine concentration of renal venous blood seems to account for only a small proportion of total endogenous creatine formation at estimated rates of renal and splanchnic blood flow. However, the sharp rise in creatine concentration across the splanchnic area as determined from hepatic vein blood is sufficient to account for the known rate of endogenous creatine formation. It would appear that creatine is formed primarily in the splanchnic area, probably by the liver. At least some of the creatine synthesized in that area seems to come from non-glycocyamine precursors.

Studies of Vitamin B_{12} in Human Serum. WALTER G. UNGLAUB and HAROLD L. ROSENTHAL, New Orleans, Louisiana. (Introduced by Grace A. Goldsmith).

A method for the assay of vitamin B_{12} in serum, employing Lactobacillus leichmannii, has been developed and the serum concentrations of this vitamin have been studied in normal subjects and in patients with macrocytic anemia.

The average serum concentration of vitamin B_{12} in twelve normal subjects in the fasting state was 0.21 millimicrogram per ml. (standard deviation $\pm$ 0.08 millimicrogram). In three normal subjects maintained on

their usual diets, serum levels obtained at four hour intervals during a 24 hour period ranged from 0.11 to 0.42 millimicrograms per ml. with no constant pattern of variation.

Oral administration of 500 micrograms of vitamin B_{12} to two normal subjects and of 1000 micrograms to a third was followed by slight increases in serum vitamin B_{12} which were not considered significant.

In one patient with pernicious anemia serum vitamin B_{12} was 0.07 millimicrograms per ml. during fasting and rose following oral administration of 500 micrograms of vitamin B_{12} to a maximum of 0.32 millimicrograms per ml. after four hours, declining to 0.19 millimicrograms after 12 hours. A definite hematopoietic response followed this procedure.

Urinary excretion of vitamin B_{12} in the four subjects who received oral doses was not significantly increased.

One patient with macrocytic anemia and extensive neurological changes compatible with subacute combined sclerosis excreted quantities of vitamin B_{12} in the urine which were thirty times that found in normal subjects. In this patient, serum vitamin B_{12} during fasting varied from 0.12 to 0.73 millimicrograms per ml. Following parenteral administration of 100 micrograms and later of 500 micrograms of vitamin B_{12}, serum concentrations rose to 3.84 and 24.6 millimicrograms per ml. respectively at the end of one hour, were moderately elevated after 8 hours and slightly above the pre-test level after 24 hours. Implications relative to vitamin B_{12} metabolism will be discussed.

Fate of Intravenously Injected Labelled Gamma Globulin in the Normal Subject and in Patients with Rheumatoid Arthritis. J. H. VAUGHAN, A. ARMATO, J. GOLDTHWAITE, P. BRACHMAN, C. B. FAVOUR and T. B. BAYLES, Boston, Massachusetts. (Introduced by Eugene Eppinger).

Separate pools of gamma globulin from normal subjects and patients with rheumatoid arthritis, obtained as Fraction II by Cohn's method of alcohol fractionation, have been labelled with I^{131}. Each pool has been injected into four normal subjects and four patients with active rheumatoid arthritis. The disappearance of the material from the plasma of the recipients has been traced; the localization of radioactivity among the plasma proteins of the recipients has been defined, and in some instances a quantitative evaluation of the radioactivity appearing in the urine has been made.

In five experiments the average half-life of injected gamma globulins in normal subjects was found to range from 7.0 to 8.8 days. In each instance simultaneously injected rheumatoid arthritis patients evidenced a shorter half-life, the average difference being 1.8 days. There were no data to suggest that the gamma globulins from rheumatoid patients differed structurally from those of normals, in that none of the recipients appeared to differentiate between the two. Fractionation of recipients' plasmas immediately after and at one and two week intervals after injection revealed all radioactivity to be retained within the globulins of Fractions I + II + III, with no significant amount in Fractions IV + V. Urinary radioactivity was not associated with protein and generally was about 75% of the amount that would be expected to appear from a consideration of the plasma decay curve. Stool analyses were not done.

In three experiments the rate of disappearance of the injected material was significantly accelerated in both normal subjects and rheumatoid arthritis patients, apparently on the basis of partial denaturation incident to the ioslation and labelling procedures.

Scanning of the accessible surface of the body with a beta-gamma survey meter showed no evidence for organ localization of the injected material, neither that from normal nor that from rheumatoid donor pools.

Turn-over Rates of Serum Proteins in Cirrhotic Man. WADE VOLWILER, KENNETH FREMONT-SMITH and MARION P. MACMARTIN, Seattle, Washington. (Introduced by Robert S. Evans).

Following oral administration of S-35 tagged dl-cystine, turn-over rates of serum total protein, total globulins, albumin, gamma globulin, and Cohn fraction I + III have been measured in 3 patients with advanced cirrhosis and in 2 normal subjects.

Apparent average values obtained for molecular half-life and per cent of the total fraction in the plasma pool turned over daily were as follows: *total protein,* cirrhosis 22.4 days or 3.5%, normals 9.6 days or 7.3%; *albumin,* cirrhosis 20.6 days or 3.4%, normals 17.1 days or 6.0%; *total globulins,* cirrhosis 11.8 days or 5.9%, normals 7.5 days or 10.7%; *gamma globulin,* cirrhosis 25.0 days or 3.1%, normals 27.0 days or 2.6%; *Cohn fraction I + III,* cirrhosis 13.5 days or 7.7%, normals 4.5 days or 16.2%.

Because of the small number of subjects and the degree of variation observed, the apparent discrepancies between cirrhotics and normals may not be significant. It is believed these apparent half-times are falsely lengthened by isotope reutilization. Attention is called to the very rapid turn-over time in normals of Cohn fraction I + III, which contains about 60% of the protein-bound lipids.

Inhibition by Cortisone and ACTH of the Fever-Producing and Lymphopenic Action of Influenza Virus. ROBERT R. WAGNER and F. DOUGLAS LAWRASON, New Haven, Conn. (Introduced by Edward C. Curnen).

The intravenous injection of influenza virus in rabbits is followed by a characteristic febrile and lymphopenic response. These manifestations of toxic action are not noted when animals are given a second injection of the same virus 24 hours later. The febrile response can be prevented by premedication with antipyrine, but the lymphopenia is unaffected by this drug.

When rabbits were given a single intramuscular injection of cortisone (15 mg.) they developed lymphopenia which was less marked than that produced by influenza virus. Within 12 hours following the administration of cortisone the absolute number of circulating lymphocytes

returned to pre-injection levels. When tested after this interval, these animals showed partial to complete refractiveness to both the pyrogenic and lymphopenic actions of the PR8 and Lee strains of influenza virus. A similar, but less marked, tolerance to the toxic effect of these viruses was produced when ACTH (12 mg.) was given by the intramuscular or intravenous route 6 to 24 hours prior to virus challenge. Exposure to cold or the injection of lipo-adrenal extract did not induce a similar refractory state. Neither cortisone (15 mg.) nor ACTH (12 mg.) affected the febrile response of rabbits to a minimal pyrogenic dose of typhoid vaccine.

When ACTH (6 mg.) was given intravenously to rabbits there followed in 2 to 4 hours a reduction in the number of circulating lymphocytes amounting to 60 per cent of the pre-injection mean level. This action of ACTH was not demonstrable in animals which had received a large intravenous dose of the Lee strain of influenza B virus 24 hours previously.

Effects of Oral and Intravenous Gastric Juice upon Vitamin B_{12} Activity in Pernicious Anemia. RALPH O. WALLERSTEIN, JOHN W. HARRIS and ROBERT F. SCHILLING, Boston, Mass. (Introduced by George P. Berry).

Observations were made in patients with pernicious anemia to determine (1) whether there was evidence that parenterally administered gastric juice could render Vitamin B_{12} hematopoietically more active, and (2) whether the potentiating effects of orally administered gastric juice upon Vitamin B_{12} involved a stoichiometric relationship between these substances.

A pool of gastric juice (containing no Vitamin B_{12}) was obtained from normal donors, brought to neutrality with 20% NaOH, rendered sterile by passage through a Berkefeld filter, and stored frozen at $-32°C$.

(1) Five patients given incubated mixtures of 0.33 μg Vitamin B_{12} and 10 ml inactivated gastric juice daily intravenously made moderate reticulocyte responses. When, in a second period, identical quantities of active gastric juice and of vitamin B_{12} were given, 4 patients made no second reticulocyte peaks; the fifth patient showed a second rise. In a third period, all 5 patients made marked reticulocyte responses to 1 μg Vitamin B_{12} daily intramuscularly.

(2) Two patients were given daily oral mixtures of 1 μg Vitamin B_{12} and 10 ml gastric juice in an initial period and made moderate reticulocyte responses. Second reticulocyte responses appeared, when, with the same amount of gastric juice, the Vitamin B_{12} was increased to 5μg daily. Two other patients were given daily oral mixtures of 1 μg Vitamin B_{12} and 10 ml gastric juice in an initial period. They made moderate reticulocyte responses when, in a second period, wih the same amount of Vitamin B_{12}, the gastric juice was increased to 25 or 50 ml daily.

These findings suggest that because hematopoietic potentiation of Vitamin B_{12} by gastric juice was not shown to take place parenterally, an enteral effect leading merely to enhanced absorption of Vitamin B_{12} is involved upon oral administration, but without a stoichiometric relationship between Vitamin B_{12} and gastric (intrinsic) factor.

Measurement of Extracellular Fluid with Radiosulfate. MACKENZIE WALSER, DONALD W. SELDIN * and ARTHUR GROLLMAN, Dallas, Texas.

For use in the measurement of ECF, tracer ions have the theoretical advantages of rapid diffusion and freedom from osmotic effects. Most ions studied, including sulfate, have shown progressive cell penetration. Sulfate penetrates cells very slowly, however, although it leaves the plasma as rapidly as sodium. The volume of distribution of radiosulfate after attainment of equilibrium across the capillary membrane was therefore investigated as a measure of ECF.

Following intravenous injection of radiosulfate in normal subjects the plasma concentration decreased at a simple exponential rate after the first 15–20 minutes in man (25–30 in dogs), for as long as 5 hours. The volume of distribution, calculated as the amount injected minus the amount excreted divided by the plasma concentration, increased linearly with time at the same rate in human subjects and dogs. After bilateral nephrectomy in dogs the volume of distribution increased only 6% between 25 minutes and 5 hours.

Preliminary results of muscle analysis following administration of radiosulfate to nephrectomized dogs indicate that radiosulfate space of muscle remains less than chlorine space of muscle.

It was concluded that equilibrium of distribution across the capillary membrane was virtually complete in about 18 minutes in man and about 27 minutes in dogs. In support of this hypothesis, analysis of the early arterial curves revealed that a single rate (0.27–0.28 in man) accurately described the initial disappearance.

Estimates of ECF by this means gave values averaging 17% of body weight in man and 21% in dogs. Simultaneous radiosulfate and inulin or mannitol spaces corresponded in most cases, and changed by similar amounts when ECF was altered. Results were reproducible within 0.3 liter. Changes induced by water loading, water deprivation (in diabetes insipidus), and other means equalled simultaneous changes in chloride space.

Demonstration in Man of Type Specific Immunity to Streptococcal Infections. LEWIS W. WANNAMAKER and FLOYD W. DENNY, Cheyenne, Wyoming. (Introduced by Charles H. Rammelkamp, Jr.).

Although animal experiments indicate that immunity to Group A streptococcal infections is type specific, the nature of resistance to such infections has not been well defined in man. In an attempt to clarify the role of type specific immunity in human disease, the spread of Group A streptococci was studied in a group of 43 men housed in a single barrack. Type 14 bacteriostatic antibodies were demonstrated in 13 individuals. Twenty-two men were considered to have no antibodies to this type. The remaining 8 men were carriers of Group A streptococci.

Five harbored type 14 organisms and one each was a carrier of types 1, 5, and 6. Bacteriological and clinical observations were made three times a week for a period of six weeks.

In both the group with antibodies and the group without antibodies approximately seventy-five per cent of individuals harbored streptococci in the nose at some time during the observations, and in approximately half of both groups type 14 organisms were found in the nose. Although this suggests that the opportunity for infection was equal in both groups, the incidence of parasitism of the nose and throat, clinical disease, and immunological response was related to the presence of type specific antibodies. Seven of the 22 men without antibodies to type 14 streptococci were infected with this organism but none of the men with demonstrable antibodies were parasitized by this type. In contrast, parasitism with a heterologous type of streptococcus occurred in 6 of the 22 men without antibodies and in 6 of the 13 men with antibodies. Concomitant symptoms and signs of an upper respiratory infection developed in approximately half of the individuals parasitized. These observations suggest that the antibodies measured have a protective action against infection with streptococci of a homologous type but no protective action against infection with streptococci of heterologous types.

The Use of Modified Globin as a Protein Supplement in Normal Individuals. C. WATERHOUSE, L. D. FENNINGER and E. H. KEUTMANN, Rochester, New York. (Introduced by Howard B. Slavin).

Experimental studies in animals have indicated that the protein moiety of hemoglobin can readily be utilized after erythrocytes have been broken down, and the resultant synthesis of such complete proteins as plasma albumin has been demonstrated.

Modified human globin, with a molecular weight of about 34,000 has been prepared by partial hydrolysis of erythrocytes.[1] Four patients, receiving a basic diet low in protein but adequate in calories, were given this substance intravenously for 6 to 10 days in amounts of 32 to 64 grams daily, following suitable control periods. Metabolic studies were carried out in a manner similar to that used previously with human serum albumin. The results were not entirely comparable because the desired caloric intake was not maintained in any patient after several doses of globin. Nevertheless, certain fundamental differences between the metabolism of globin and albumin are apparent.

There was no sustained increase in the plasma osmotic pressure, such as is seen when albumin is given. The body weight and hematocrit were constant, and no significant accumulation of globin in the blood occurred even after repeated injections.

Globin was excreted in the urine in considerable quantities each day. Urinary excretion did not increase with repeated administration but depended on the quantity given on any particular day and represented about 25 per cent of that injected.

About 30 per cent of the globin administered appeared within 24 hours in the urine as nitrogen in excess of globin excretion. Good utilization of the remaining globin (as determined from the phosphorus balance corrected for calcium exchange) occurred readily when the caloric intake was adequate.

In contrast to albumin with a half disappearance time of about 5 days, the breakdown of modified globin is rapid. The amino acid components then seem to be handled as might any injected amino acids, subject to the general laws of protein metabolism.

[1] Prepared by Sharp and Dohme, Incorporated.

Effect of Arteriovenous Fistula on the Circulation. RENÉ WÉGRIA,* CHARLES W. FRANK, HSEUH H. WANG and ROBERT MILLER, New York, N. Y.

Dogs were anesthetized with chloralose and their chest was opened through a midsternal incision. Under artificial respiration, two rotameters were inserted into the arterial side of the systemic circulation. The first one, inserted according to a technique previously described (Proc. Soc. Exp. Biol. & Med., 74: 551, 1950), recorded the output of the left ventricle with the exception of the amount of blood flowing through the arteriovenous fistula which was recorded by the second rotameter. The magnitude of the fistula established between the root of the aorta and the proximal end of one of the femoral veins could be adjusted by a tunnel-clamp. Mean arterial blood pressure was recorded simultaneously with the amount of blood flowing through the fistula and the "body flow," i.e. the output of the left ventricle minus the fistula flow. The fistulas were established progressively, maintained for five minutes, then suddenly occluded. The results observed can be summarized as follows:

With "small" fistulas, i.e. fistulas accommodating a blood flow of up to 20 per cent of the control cardiac output, generally neither the "body flow" nor the mean arterial blood pressure was modified and the cardiac output was increased by an amount equal to the fistula flow. With "large" fistulas, i.e. fistulas accommodating blood flow up to around 60 per cent of the original cardiac output, the increase in cardiac output which occurred was greater than with the "small" fistulas, but the "body flow" fell below its control level; as shown by an increase in the total peripheral resistance, vasoconstriction occurred and the mean arterial blood pressure fell only slightly. With "very large" fistulas, i.e. amounting up to around 90 per cent of the control cardiac output, the increase in cardiac output was still greater; the "body flow" decreased markedly and, despite a marked vasoconstriction, an important decrease in mean arterial blood pressure occurred.

Effect of Desoxypyridoxine on Acute Lymphatic Leukemia of Adults. DAVID R. WEIR, Cleveland, Ohio. (Introduced by Joseph T. Wearn).

Desoxypyridoxine, a potent antagonist of pyridoxine, is capable of producing pyridoxine deficiency in animals and

man. Necessity for a pyridoxine-deficient diet can be eliminated by administration of larger amounts of desoxypyridoxine. Thus, desoxypyridoxine administration to mice ingesting non-deficient diets results in the characteristic hematologic changes of granulocytosis, lymphopenia, and myeloid metaplasia in the spleen and these changes can be prevented or reversed by pyridoxine administration. Reversal of the granulocyte : lymphocyte ratio occurs in other conditions, including cortisone administration. The pyridoxine deficiency changes have been induced in adrenalectomized mice, however, indicating that they are not mediated through the adrenal glands.

In mice having a mixed type of transplanted leukemia, pyridoxine deficiency increased the severity of the disease with excess granulocyte production and decreased survival time.

Five adult patients having acute lymphatic leukemia were maintained on a normal diet and given desoxypyridoxine in divided oral doses of up to 1.4 gm. daily for 80 days. Toxic effects were not observed nor did seborrheic dermatitis appear. In one patient the leukocytes decreased from 90,000 to 8,000 per cmm. with almost complete disappearance of abnormal cells from the blood and with a rise in hemoglobin. Marked regression of enlarged lymph nodes and spleen occurred. In a second patient the leukocytes decreased from 120,000 to 7,600 per cmm. and moderate regression of nodes occurred. In a third patient there was regression of nodes and improvement of clinical condition without significant hematologic change. Two other patients who were *in extremis* died shortly after the institution of treatment.

Thus, pyridoxine deficiency is associated with specific hematologic changes. Preliminary trial suggests that induced pyridoxine deficiency may be of some use in the treatment of certain human acute leukemias but its effectiveness requires further evaluation.

Augmentation by Cortisone of Nitrogen Mustard-induced Leukopenia. AUSTIN S. WEISBERGER and BENNETT LEVINE, Cleveland, Ohio. (Introduced by R. F. Parker).

Cortisone and ACTH are therapeutically effective in certain conditions in which nitrogen mustard (HN_2) is beneficial. Because granulocytosis occurs following administration of the hormones, it has been suggested that they might be capable of alleviating the leukopenia induced by HN_2. However, in investigating the role of the spleen in HN_2-induced leukopenia, it was observed that HN_2-treated rabbits in which sham operation with simple abdominal incision was performed developed greater leukopenia than unoperated controls. This indicated that the leukopenia was greater under conditions of stress.

Administration to rabbits of 10 mg. of cortisone per Kg. body weight for 2 days prior to, or 5 mg. per Kg. for 3 days after, the injection of HN_2 did not prevent the characteristic severe leukopenia but resulted instead in a significant increase in the degree of leukopenia and neutropenia. In animals receiving both HN_2 and cortisone the neutrophils decreased to 3% of the initial value, while in animals given only HN_2 there was a decrease to 22% of the initial value. Similarly, cortisone administration lessened the granulocyte protective effect obtained when L-cysteine is administered just prior to injection of HN_2.

The mechanism by which cortisone administration and stress augment the severity of the HN_2-induced leukopenia is not known. We have proposed that the protection against this leukopenia afforded by the prior administration of L-cysteine and closely related compounds results from competition by these compounds with HN_2 for some substance necessary for normal leukopoiesis. Cortisone has been shown to inactivate SH-containing compounds so that the increased severity of the leukopenia produced by administration of both cortisone and HN_2 may be the result of cumulative interference with the metabolism of certain substances which normally contain or combine with SH-containing compounds and which are necessary for normal leukopoiesis.

The Site of the Saluretic Action of an Organic Mercurial Compound. L. G. WELT, A. V. N. GOODYER, J. H. DARRAGH, W. A. ABELE and W. H. MERONEY, New Haven, Conn. (Introduced by John P. Peters).

The current concept of renal physiology implies that proximal tubular reabsorption of solutes is active and that the reabsorption of water follows as a passive consequence. The extent of proximal reabsorption of solute determines the volume of fluid, isosmotic with glomerular filtrate, delivered to the distal tubule, where reabsorption of water is active and largely controlled by antidiuretic hormone. With ordinary volumes of distal tubular fluid, maximum reabsorption of water is limited by a total solute concentration approximating 1,400 mOsm./L. However, when the volume of fluid delivered to the distal tubule is excessive, the final urine may be dilute despite continuous maximal antidiuretic activity.

Thus, a decrease in *proximal* tubular reabsorption of salt would be expected to promote the excretion of large volumes of dilute urine despite maximal antidiuretic activity. A decrease in *distal* reabsorption of salt, however, would promote only a modest increase in volume of a urine with persistently high total solute concentration.

Three normal hydropenic male subjects received a continuous infusion of saline containing sufficient pitressin to insure the administration of 100 milliunits an hour. When a steady state had been achieved, 2 cc. of thiomerin were administered. This promoted an increase in the rates of excretion of water, sodium, and chloride, little or no change in ammonia and urea, a decrease in potassium, and no essential change in the clearance of endogenous creatinine. The augmented rate of excretion of salt was accompanied by a decrease in the concentration of each of the measured urinary solutes including sodium and chloride.

These studies imply that the influence of mercury with respect to the tubular reabsorption of salt *must* be proximal, although it *may* be distal as well.

Coagulation Changes in the Isolated Venous Segment of the Dog. STANFORD WESSLER, Boston, Mass. (Introduced by Benjamin Alexander).

The mechanisms involved in the initiation and propagation of intravascular clotting remain obscure. Studies to elucidate certain aspects of this problem have been carried out in the dog. Isolated blood-filled venous segments were prepared in 35 animals so that the blood was completely stagnant and the vascular segment entirely cut off from the general circulation. In these segments containing 1–2 cc. of blood, grossly visible clots rarely appeared within 60 minutes but always formed within 120 minutes. In six dogs the prothrombin concentration of the blood in these segments was determined two minutes to nine hours after isolation of the segment. By the two-stage method, prothrombin concentration remained unchanged for four hours despite evident deposition of a clot. Thereafter, prothrombin decreased slowly, but did not disappear completely in nine hours. The slow rate of platelet disappearance and the one-stage prothrombin values, which paralleled the two-stage results, confirmed the marked deceleration of clotting afforded by endothelial tissue.

In contrast, dog serum and purified SPCA (an accessory prothrombin conversion factor in serum) uniformly produced a clot within three minutes after their injection into the segment. This is the first time that SPCA, which evolves as a consequence of clotting, has been shown to act as a clot-promoting agent in an endothelial-lined segment. Clots produced in this manner do not adhere to the endothelial wall during the first 48 hours after their formation and therefore act as emboli when the continuity of the venous segments with the general circulation is restored.

The use of this approach may facilitate study of the earliest phases of the coagulation sequence and should permit study of clot propagation and clot adherence under a variety of altered physiologic states.

Production of Water Retention and Acute Hyponatremia Without Sodium Loss by Administration of Pitressin Tannate to Patients in Congestive Heart Failure. R. E. WESTON, I. B. HANENSON, E. R. BORUN, J. GROSSMAN and M. WOLFMAN, New York, N. Y. (Introduced by Louis Leiter).

Previous studies have indicated that intensification of congestive failure by severe respiratory infection or inadequate digitalization may lead to water retention and acute hyponatremia without sodium loss in cardiac patients on low salt diets. To evaluate the role of increased ADH activity in this phenomenon, the effect of daily intramuscular injections of Pitressin Tannate in Oil (PTO) on metabolic balances of water, sodium, potassium, chloride, nitrogen and phosphorus, corresponding blood chemistries, and eosinophile counts of seven rheumatic cardiacs in chronic congestive failure was studied.

On 5 units of PTO daily for 3 to 7 days, four cardiacs, maintained on 2000–2700 ml. fluid and 15 m.eq. sodium intakes, had reduced urine flows, 1.8 to 4.9 kgm. weight gains, and 17–27 m.eq./l. decreases in serum sodium and chloride concentrations. Concurrently, they developed increased edema, dyspnea and venous pressure, eosinopenia, and further reduction in urinary sodium output. When restudied on higher salt intakes, two of these patients exhibited greater electrolyte retention and, consequently, smaller decreases in serum sodium and chloride levels during PTO administration. Within 24–48 hours after discontinuing PTO, water diuresis began. In 24–96 hours, pre-pitressin status was restored, and urinary sodium and chloride outputs increased to above levels.

Three cardiacs manifested no significant changes on 5 units of PTO daily. This dose, twice daily, promptly produced the response described above in two of these patients. Despite continued administration of PTO, after 16 days one of these had spontaneous water diuresis accompanied subsequently by natriuresis.

In one non-cardiac hyperthyroid patient on a full salt intake, 5–15 units of PTO daily for 9 days produced no changes.

Calculated fluid and electrolyte shifts, and the significance of water retention in the pathogenesis of acute hyponatremia in congestive failure, will be discussed.

Calcium Loss in Paralytic Poliomyelitis and Its Quantitative Relationship to the Development of Demonstrable Osteoporosis. G. DONALD WHEDON, New York City, N. Y. (Introduced by Ephraim Shorr).

Metabolic balance studies were carried out for 6 to 10 months in 9 patients with acute anterior poliomyelitis ranging in age from 17 to 39 years. Paralysis was generally marked and extensive. Studies on 4 of the patients were begun early in the acute phase. As soon as possible constant diets were instituted, the calcium intake being 920 mgm. daily. Standard methods of therapy were used.

Progressive increase in both urinary and fecal calcium was noted. The maximum urinary calcium (for a six-day metabolic period) ranged from 391 to 789 mgm./day, the mean being 572 mgm./day, and occurred on the average 5 weeks after onset. Urinary calcium began to decline slowly before ambulation was begun but remained above 250 mgm./day for 5½ months. Excellent background for urinary tract calculus formation was afforded by extensive increases in urinary phosphorus as well as calcium, accompanied by little change in urinary volume and citric acid.

The mean maximal negative calcium balance (−0.540 gm./day) occurred during the ninth week. Mean duration of negative balance was 7 months, the patients having been recumbent for 6 months on the average.

Mean serum calcium during the first four months was 10.8 mgm.%; in two patients, however, one-third of the values were above 11.5 mgm.%.

On independent evaluation of monthly x-rays by a radiologist unaware of the metabolic data, initial evidence of osteoporosis, usually patchy demineralization and coarsening of trabecular markings in the lower extremities regional to joints and most often in necks and distal ends of the femora, appeared approximately three months after onset with average loss of 2% of estimated total

body calcium. Total calcium losses for the 7 months of negative balance averaged 4% and ranged up to 8.5% without detectable x-ray changes in the spine.

The Effect of Intravenous Pitressin on the Renal Excretion of Water and Electrolytes in Congestive Heart Failure and in Liver Cirrhosis. ABRAHAM G. WHITE and GEORGE RUBIN, New York, N. Y. (Introduced by Ernst P. Boas).

An infusion of 5% glucose in water was administered intravenously at 10 cc. per minute to seven control subjects, ten cardiacs, and five patients with liver disease (four with decompensated liver cirrhosis and one with infectious hepatitis), and the resulting water diuresis studied. While the glucose infusion continued and after the urine flow reached a plateau, 0.57 mU/kg of Pitressin was injected intravenously into the seven controls, into six of the cardiac patients and into the five hepatic patients.

Following onset of the infusion the average time required for attainment of peak diuresis in the control subjects, cardiac patients, and liver patients was: 85.1 ± 32.3, 99.1 ± 32.1, and 88.6 ± 27.2 minutes, respectively. The average peak urine flows attained during the hydration periods were: 12.0 ± 2.7, 6.7 ± 3.3, and 11.9 ± 3.0 cc/minute for the three groups.

There was no significant difference in the duration of, and intensity of, Pitressin antidiuresis under these experimental conditions in three groups of patients, except for two of the cardiacs who demonstrated an enhanced water antidiuresis. In the dosage administered, Pitressin did not exert any significant effect on the urinary output of sodium, chloride or potassium. As for the effect of Pitressin on the concentrations of electrolytes in the serum, the only consistent change noted was that of the serum sodium which tended to fall during Pitressin antidiuresis in the five patients with liver disease. The two cardiac patients who showed the augmented Pitressin effect also had the lowest concentrations of serum sodium (121.9 and 130 mEq/L), but a causal relationship remains to be established.

In summary, these data offer no conclusive evidence for any deficiency in the mechanisms of inactivation of Pitressin administered in physiological dosage to controls, cardiacs or patients with liver disease.

PTC Factor: A New Plasma Thromboplastin Component Distinct from the Anti-Hemophilic Factor. SIDNEY G. WHITE, PAUL M. AGGELER,* MARY BETH GLENDENING, ERNEST PAGE, TILLIE B. LEAKE and GEORGE BATES, San Francisco, California.

A 16-year-old male was found to have normal amounts of all known coagulation factors, no increase in circulating anticoagulants, markedly prolonged whole blood coagulation time and defective prothrombin utilization. The defect was corrected by accelerator-free tissue thromboplastin and by "platelet-free" normal and hemophilic plasma, but not by antihemophilic globulin (Cohn's Fraction I) (AHG). These findings indicate the absence from his plasma of a previously undescribed coagulation factor, the plasma thromboplastin component (PTC), which is biologically as important as AHG in the production of thromboplastin in blood.

PTC is adsorbed from oxalated plasma by all known prothrombin adsorbants and by Seitz filtration. It is associated with the plasma euglobulins, and unlike AHG is present in the serum. It is most stable in the neutral ranges and is heat stable. It can be separated from previously described coagulation factors and concentrated as follows: Oxalated "platelet-free" plasma is heated to 56°C. for 5 minutes to destroy prothrombin, spca, Factor V, and labile factor. The coagulated fibrinogen is removed by centrifugation. PTC is separated from AHG by adsorbing it on $BaSO_4$, from which it is eluted with 5.8% sodium citrate. The thrombin-free eluate is dialyzed against 0.9% NaCl and lyophylized.

The patient's PTC fraction assayed against his own blood, using a "two-stage" prothrombin utilization test, is inert, while that obtained from normal, hemophilic, thrombocytopenic, and dicumarolized hypoprothrombinemic plasma is significantly active.

PTC is not a platelet factor, AHG, or a "complete" thromboplastin, such as that derived from tissue sources, since it will not correct the defect in "platelet-free" normal plasma or platelet-rich hemophilic plasma. It is not the plasma thromboplastin of Chargaff and West, since the supernatant from normal plasma centrifuged at 20,000 r.p.m. for 150 minutes completely corrects the patient's defect.

Hemodynamic Changes in Patients with the Eisenmenger Complex. K. WILLIS, T. J. REEVES, E. BOOTH, T. A. LOMBARDO, C. LOMBARDO and S. TULUY, Birmingham, Alabama. (Introduced by T. R. Harrison).

Findings by means of cardiac catheterization and electrokymographic studies of the same patients have been as follows:

(1) The interventricular shunt is indicated by characteristic reciprocal paradoxic movement of the ventricular borders. These indicate a left-to-right shunt (mainly in systole) and a right-to-left shunt (mainly in diastole).

(2) Paradoxic border movement due to volume changes produced by intraventricular shunts must be distinguished from paradoxic movement due to shape changes which are a normal phenomenon. Criteria for such distinction are presented.

(3) The experience to date points toward distinctive electrokymographic records in patients with the Eisenmenger complex. However, it is as yet uncertain whether similar border movements are present in other types of congenital malformation associated with interventricular septal defects.

Dynamic Response Characteristics of Manometer Systems Necessary for Adequate Recording of Intravascular Pressure Pulses in Man. EARL H. WOOD,* Rochester, Minnesota.

Practical considerations necessitate the use of small needles or long narrow bore plastic tubes for direct

recording of pressures in the vascular system of man. Minimum cross-sectional dimensions and maximal length of needles or tubes suitable for this purpose are governed by the dynamic response characteristics of manometer systems necessary for recording of the dynamic components of practical importance in the pulse wave. There are, however, no direct data in the literature concerning just how much the dynamic response of manometer systems can be reduced without producing significant distortion of recorded pulse waves.

Intrabrachial arterial pressure was photographed continuously while intraradial pressure was simultaneously recorded from the same arm with 5 different manometer systems of widely different dynamic response. Differences in radial systolic pressure and pulse contour could not be detected in 11 normal and 5 hypertensive patients when manometer systems with uniform response out to 6 cps and above were used. However, a decrease in radial systolic pressure averaging 4 mm. Hg (P value 0.01) was detected in recordings from a manometer system with uniform response to 2 and a 90 per cent response at 4 cps. None of the manometers changed diastolic or mean pressure.

Similar results were obtained in studies of aortic pressure pulses using Peterson catheters attached interchangeably to capacitance and strain gauge manometers and in studies of intracardiac pressures by means of catheters tipped with miniature manometers.

It is concluded that dynamically adequate manometer systems can be assembled even when miniature (1 mm. O.D.) 1 meter length catheters are used. Amplifier instability and artefacts due to rhythmic catheter motion are much more frequent sources of non-recognized errors in pressure recordings via catheters than are the amplitude and phase distortion characteristics of the manometer systems used.

The Normal and Modified Erythrocyte: Observations of Certain Phagocytable Characteristics. CLAUDE-STARR WRIGHT, MATTHEW C. DODD, JOSEPH A. BASS and NOREEN G. BRANDT, Columbus, Ohio. (Introduced by Charles A. Doan).

There has been little correlation between *in vivo* and *in vitro* tests for qualitative abnormalities in the erythrocytes. New *in vitro* means have therefore been sought to determine the earliest minimal changes in erythrocyte integrity. This has been partially accomplished by the determination of phagocytic indices utilizing normal and modified erythrocytes as test cells. Macrophages cultured from rabbit spleens in the presence of homologous serum were the stock phagocytizing cell. Normal and modified erythrocytes were added to tissue cultures of 3–5 day old macrophages. Species differences between the rabbit and the human were not apparent since normal erythrocytes from both were phagocytized by only 0–10% of the rabbit macrophages in the control cultures. Cells from healthy adult humans were used to establish these base lines.

Studies to date indicate that the following modified erythrocytes are more easily phagocytized than are normal cells: (1) trypsinized RBC, (2) sensitized RBC, (3) RBC from patients with hereditary spherocytosis and acquired hemolytic anemia. Phagocytosis of erythrocytes from rabbits receiving 700r total body irradiation and tested over 27 days after irradiation was not increased over the normal.

Treatment of Pemphigus with Potassium Para-Aminobenzoate. CHRIS J. D. ZARAFONETIS and JOHN SHAW, Philadelphia, Pennsylvania. (Introduced by Arthur C. Curtis).

Prolonged administration of large doses of potassium para-aminobenzoate (KPAB) has been shown to be beneficial in a variety of clinical disorders. Favorable results in dermatitis herpetiformis suggested a trial of KPAB therapy in another bullous condition, namely, pemphigus. The purpose of this report is to present briefly the results achieved with KPAB in the treatment of 15 patients with various types of pemphigus. Included are 7 patients with pemphigus vulgaris, 5 with pemphigus foliaceous, 2 with pemphigus erythematodes, and 1 with ocular pemphigus.

KPAB was administered as a 10% solution, usually 30 cc. (3.0 gm.) every 3 hours for 6 to 8 doses daily; i.e., a total of 18 to 24 gm. per day. Twelve patients had improvement characterized by cessation of exudation, subsidence of blister formation, followed by epithelialization and hyperpigmentation. Plasma specimens from several patients were subjected to electrophoretic analysis by Dr. Marie Berg, who noted general improvement in the protein pattern during the period of KPAB treatment. Those patients whose disease was long-standing responded more slowly than those with relatively recent onset. The duration of therapy varied, therefore, from a few weeks to several months. In those cases where complete suppression was achieved, treatment was discontinued and the patients observed for relapse. Remissions were maintained for as long as 7 months. Two patients who had good, though incomplete, suppression on KPAB were treated with cortisone without added improvement. It is to be emphasized that KPAB is not curative. Nevertheless, its use in the management of selected cases of pemphigus appears warranted.

Hemodynamic Studies by the Opacification Technique in Patients with Mitral Stenosis and Other Cardiovascular Abnormalities. HARRY F. ZINSSER, PAUL W. EYLER, DAVID P. MCCALLIE and WILLIAM J. TUDDENHAM, Philadelphia, Pennsylvania. (Introduced by Calvin F. Kay).

In the angiocardiographic technique, individual cardiac chambers and great vessels become opacified as seen on x-ray films taken at rapid intervals following the intravenous injection of contrast material. The changes in density of a given chamber from film to film can be measured photoelectrically by a densitometer. When these density measurements are plotted against time, opacification curves can be constructed for individual chambers, and from numerous curves, a mean opacity curve can be

derived. Such curves are related to the velocity of blood flow and to the various factors governing it.

The mean *right* heart curve for normal subjects showed a rapid rise and slower fall. The entire curve was completed by six seconds. This curve was altered in various conditions which delay emptying of the right heart, for example in cor pulmonale, Ebstein's anomaly, and pulmonic stenosis.

In normal subjects, opacification of the left heart chambers and aorta showed an essentially uniform duration and intensity. The mean *left* heart curve for normal subjects began at five seconds and faded by ten seconds after injection.

In mitral stenosis, *right* heart density curves were not significantly altered except where secondary pulmonary vascular changes were proven or suspected. *Left* heart curves, however, were markedly altered. The left atrium remained sharply demarcated for long periods of time. The beginning of its mean curve was not significantly different from that of normal subjects. However, the remainder of the curve rose to high levels of opacity and did not fade until 26 seconds after the injection of opaque material. The left ventricle and aorta were poorly opacified and their mean curves differed sharply from the atrial curve. Such characteristic alterations in the duration and degree of opacification of individual heart chambers provide information of value in the selection of patients for mitral commissurotomy.

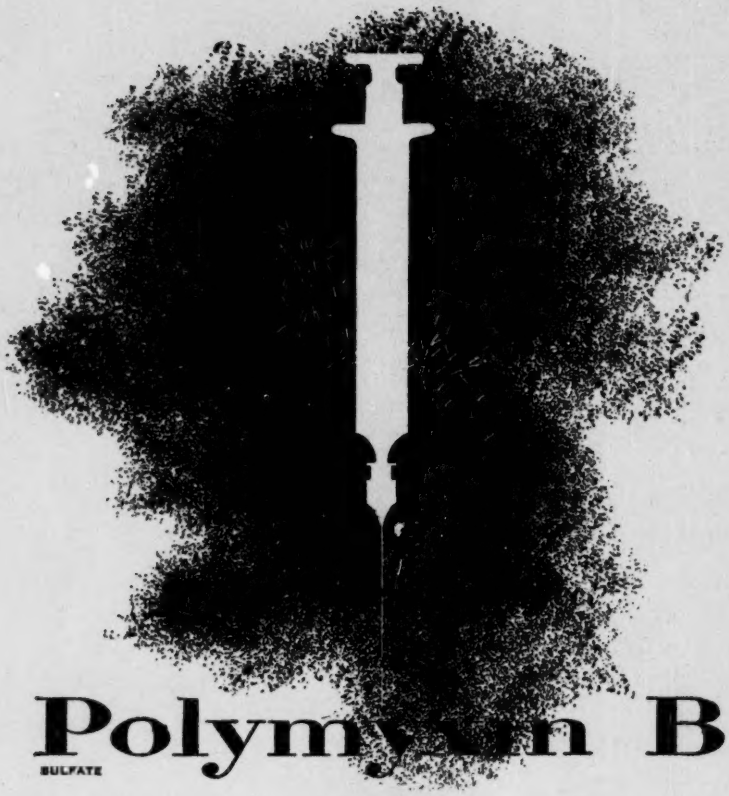
The Effect of Drugs and Anemia upon Experimentally Produced Interarterial Coronary Anastomoses. PAUL M. ZOLL and LEONA R. NORMAN, Boston, Mass. (Introduced by Mark D. Altschule).

The time required for the development of interarterial coronary anastomoses and the effects of drugs and anemia on this process were studied in 349 young domestic pigs. Coronary anastomoses of functional significance are initially absent in this animal. Acute complete ligation of the right coronary artery in 24 pigs resulted uniformly in death. Injection of the coronary arteries with colored lead-agar mass by the Schlesinger technic in these acute experiments and in 134 normal, control pigs showed uniform absence of extensive anastomoses.

Functionally significant anastomoses were demonstrated, however, following partial narrowing of the right coronary artery to 15 to 35 per cent of its original area. The same artery was subsequently occluded completely at a second operation. Functionally significant anastomoses were demonstrated by survival following final occlusion or by extensive retrograde injection of vessels distal to the occlusion. A significant effect of narrowing in stimulating anastomoses was first apparent after two days. The frequency of anastomoses increased with longer intervals, but they were not very frequent till after 12 days or more. Various vasomotor drugs were administered in the intervals between narrowing and occlusion; sodium nitrite alone increased the development of anastomoses significantly.

Thirty-nine animals were made anemic or were given vasomotor drugs for 1 to 6 weeks without preliminary coronary artery narrowing. Extensive anastomoses were found in 1 of 4 animals treated with sodium nitrite and in 2 of 14 anemic pigs. These few positive results are statistically significant in comparison with the absence of such anastomoses in 134 control hearts.

These studies delineate the rate of development of anastomoses after coronary artery narrowing. They also indicate that sodium nitrite accelerates their development and they confirm experimentally the efficacy of anemia in stimulating anastomoses that was suggested by our previous clinico-pathological studies in man.



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